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PHYSIOLOGICAL CHEMISTRY
AND
URINE EXAMINATION
—
WOLF

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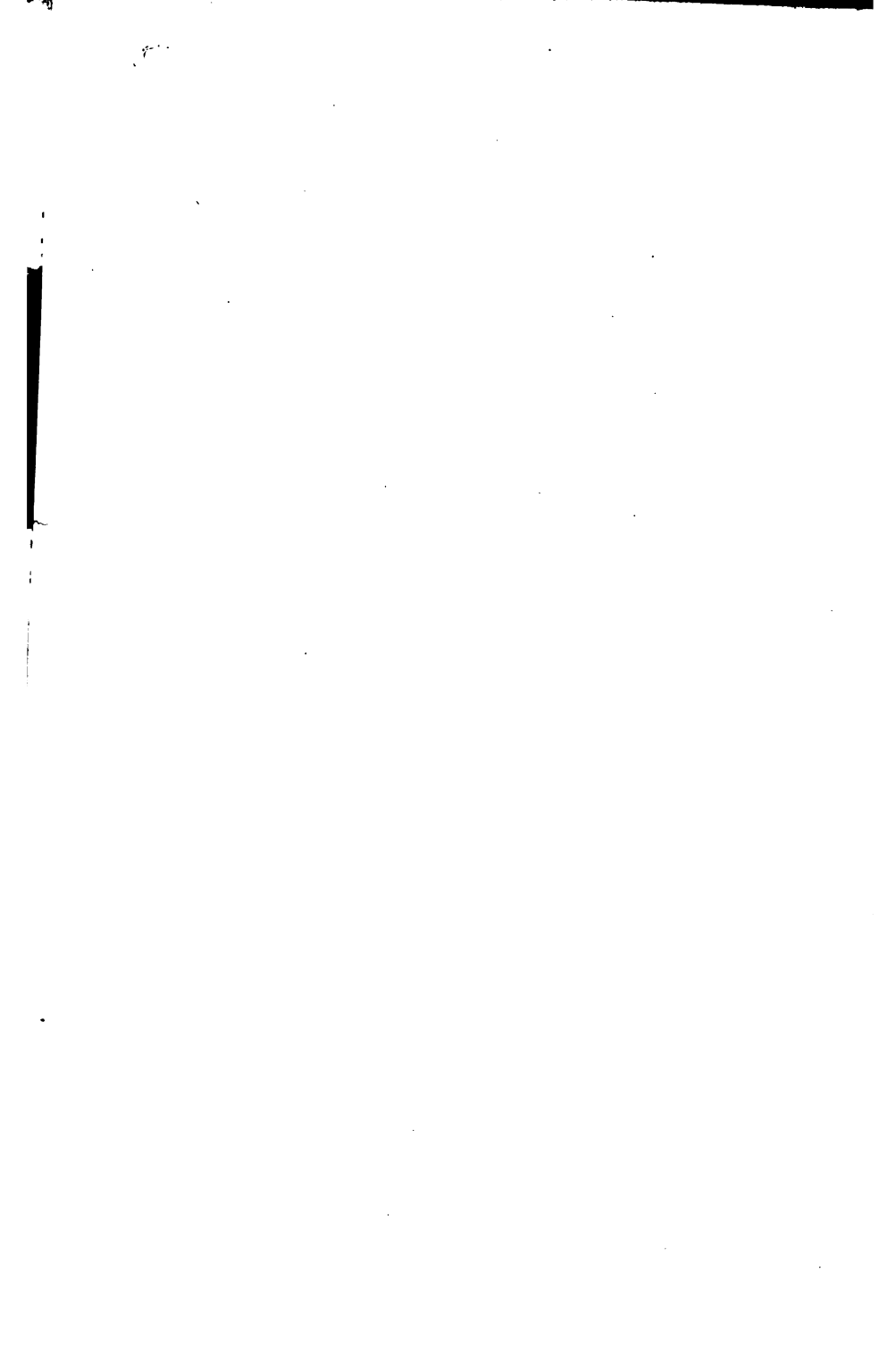
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A
LABORATORY HAND-BOOK
OF
URINE ANALYSIS
AND
PHYSIOLOGICAL CHEMISTRY

BY
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Instructor in Physiological Chemistry, Cornell University Medical
College, New York

ILLUSTRATED

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TO

F. S. and P. T.

PREFACE.

THE object of this book is to supply to students and practitioners of medicine a guide to a course in physiological chemistry and the examination of the urine and the contents of the stomach. The first part of the book is taken up with simple exercises in physiological chemistry, which it is hoped will give the student an elementary insight into the chemical side of physiological processes. While the exercises are not as full or as complete as might be desired, they are equal to the amount of time which is usually allotted to medical students in this subject.

Of the part of this book which deals with the urine and the gastric contents little remains to be said, except that, so far as possible, no tests have been given which do not rest on a good chemical foundation. The aim has been to give as few tests as possible, and these to be chosen for their suitability to purely clinical needs. No operations have been described which have not undergone a thorough trial with students in the laboratory.

In conclusion, I wish to thank Mr. Carroll D. Partridge for much help with the proof-reading, and Dr. C. N. B. Camac for the very useful diagnostic tables at the end of the book.

CORNELL UNIVERSITY MEDICAL COLLEGE,
NEW YORK, September 1, 1901.

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PRACTICAL
URINE ANALYSIS
AND
PHYSIOLOGICAL CHEMISTRY.

PHYSIOLOGICAL CHEMISTRY has to do with the examination of those processes which take place in the living organism, primarily as the result of the function of the organism itself, or secondarily by means of other or lower organisms which assist the higher one in its work.

In the consideration of the vertebrate organism, these processes may be those which take place without outside help, such as respiration, the secretory function of the various glands and glandular structures, and also the investigation of those processes which take place by means of outside agencies. Into this class falls the digestive process to a large extent.

Physiological chemistry also deals with the examination of food-stuffs, and the relation of these to nutrition and to the waste products formed in their use.

The tissues of the body are also subject to examination in this respect, not only as to their composition, but also to the changes which may occur as a result of change in nutrition or in any other condition of environment.

The first part of the exercises in physiological chemistry will be occupied with an examination of the stuffs which either enter into the composition of the animal body or are used by it as a means of nutrition or are excreted in the form of waste products.

Following this will be an examination of physiological secretions of various kinds, and lastly an examination of some of the tissues of the body.

Those substances which are met with in physiological chemistry may be roughly subdivided into inorganic and organic. In many cases these two classes of compounds are so closely in combination that it is impossible to separate them without destroying the compound itself. For this reason it is impossible to separate the inorganic part of physiological chemistry from the organic.

In the classification of the organic compounds one may go in different directions, but as a rough subdivision one may take the classification into those which contain nitrogen and those which do not. To the latter class belong the carbohydrates and the fats, while in the former class fall all the protein substances of which the albumins are the chief members. Another member of this class is important as the principal means of getting rid of waste nitrogen. This is urea, the diamid of carbonic acid.

THE CARBOHYDRATES.

The carbohydrates are classified into the monosaccharids, disaccharids, trisaccharids, and polysaccharids.

Monosaccharids.—The principal members of this class are dextroglucose (ordinary glucose or grape-sugar) and fructose (fruit-sugar).

Disaccharids.—Saccharose (cane-sugar), lactose (milk-sugar), and maltose (malt-sugar).

Trisaccharids.—Raffinose.

Polysaccharids.—Starch, the dextrins, glycogen, and cellulose.

All these compounds are chain compounds, the carbon atoms not being arranged in a ring. Inosite, which at one time was supposed to belong to this class, is, however, a cyclic compound, and cannot therefore be looked upon as a carbohydrate. All the above substances are alcohols, containing the hydroxyl group $-OH$. In addition, they either have a ketone group, $=CO$, or the aldehyd group, $-COH$.

Their action toward oxidizing agents is quite in accord with this constitution, and they therefore "reduce" them readily, and are themselves oxidized in so doing.

It is to this property that many of the tests for carbohydrates are due, and it must not be forgotten that these tests respond equally well with other reducing agents, and that a response to Fehling's test, for example, is by no means an indication that the substance which is acting on Fehling's solution

is glucose. As, however, in the case of urine the only reducing substance likely to be present in that fluid is glucose, the reduction of an alkaline copper solution is usually set down to glucose.

Glucose.—This substance is not found in the animal body in any amount under physiological conditions, but with pathological change it sometimes occurs in exceedingly large quantities, and may then readily be detected in the blood and tissues, but especially in the urine.

Glucose is formed from the more complex carbohydrates, such as starch and cane-sugar, by a process of hydrolysis, in which the sugar or starch takes up a molecule of water and breaks down into the simpler monosaccharid. This may be accomplished by means of inorganic acids, or, in fact, by any substance which on solution dissociates, giving hydrogen ions. It is also formed by certain organic ferments acting on the higher carbohydrates.

It has the property of undergoing a peculiar change when its solutions are treated with the micro-organism *saccharomyces cerevisiæ*. Under these circumstances, if the solution be not too concentrated, the glucose breaks up according to the following formulæ:



giving alcohol and carbon dioxid. This change is known as fermentation, and all the ethylic alcohol used in commerce is prepared in this way.

1. Heat a small quantity of glucose on platinum foil. Note that the substance melts and turns deep

brown. Do not heat sufficiently to char the glucose. Dissolve the residue in 1 c.c. of water. Note the deep-brown color of the solution. The substance formed by heating glucose is caramel.

2. Make a solution by dissolving 2 grams of the dry glucose in 100 c.c. of water. Taste the solution, and compare the sweetness of the solution with that of a similar solution of sugar. Test the reaction of the solution with litmus-paper.

3. Heat a small quantity of the solution with an equal amount of potassium hydroxid solution. Note the change in color, which passes through yellow to deep brown (Heller's test).

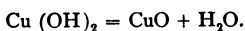
The following tests are due to the reducing power of the glucose on mild oxidizing reagents. In these reactions the aldehyd group —COH is oxidized to the carboxyl group —COOH .

4. To about 5 c.c. of the glucose solution add 1 c.c. of an ammoniacal solution of silver nitrate, made by adding dilute ammonia to silver nitrate till the brown precipitate which at first forms is dissolved. Care must be taken not to add too great an excess of the ammonia. Warm the mixture of the ammoniacal silver nitrate and the glucose. Note the formation of a mirror on the sides of the test-tube, and the precipitate of a gray color, which is due to metallic silver. This reaction is made use of in the preparation of mirrors. What is the substance formed by the oxidization of the glucose?

5. To a very dilute solution of indigo-sulfonic acid, prepared by dissolving indigo in concentrated sulfuric acid, and diluting, add a drop of glucose

solution. Then add a drop of sodium carbonate solution till the reaction of the mixture is alkaline. Warm the mixture. The indigo-blue color is discharged. Indigo-white is formed, which is colorless. On shaking the mixture with air the blue color returns. This is due to the indigo-white formed in the first instance being reoxidized by the oxygen of the air to indigo-blue.

6. Prepare some Fehling's solution by mixing equal quantities of solutions 1 and 2, and diluting with 4 parts of water. Boil the solution and add a couple of drops of the glucose solution. Note the change which takes place on boiling. Allow the tube to stand, and examine the sediment which is formed. Add a further quantity of glucose solution and boil again. The red precipitate which is formed consists of cuprous oxid, while the yellow compound which is seen on first boiling the mixture is the hydrated cuprous oxid, which loses water and is transformed into the red oxid :



7. *Böttger's Test.*—This belongs to the same class of reaction as the above. Take 1 c.c. of glucose solution and 4 c.c. of water. Add 1 c.c. of potassium hydroxid solution, and a small quantity of dry bismuth subnitrate. Boil the mixture. A black precipitate is produced of metallic bismuth.

8. *Molisch's Test.*—This test is a general one for carbohydrates, and depends on the formation of furfural when a carbohydrate is treated with concentrated sulfuric acid. Furfural gives a red

color with a solution of α -naphthol. As this test is exceedingly delicate, it is necessary to dilute the 2 per cent. glucose solution used for the foregoing tests with 10 times its volume of water. To 10 c.c. of the diluted solution, in a test-tube, add a drop of an alcoholic solution of α -naphthol. By means of a pipette allow a couple of c.c. of concentrated sul-

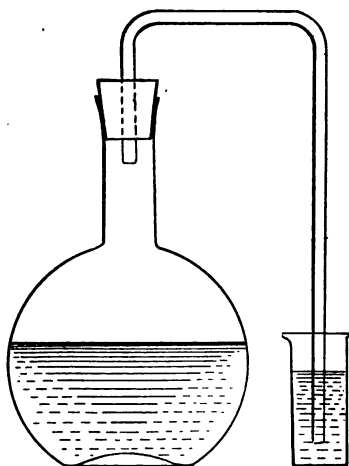


FIG. 1.—Fermentation apparatus.

furic acid to flow down the side of the test-tube. At the point of junction of the sulfuric acid and the glucose solution a red contact-ring will form.

9. *The Fermentation of Glucose.*—Take a flask holding about 250 c.c. and place in it 150 c.c. of a 2 per cent. solution of glucose, and add half a cake of compressed yeast.

Fit the neck of the flask with a doubly bent tube,

as shown in Fig. 1. The free end of the tube dips under the surface of a small beaker holding 50 c.c. of calcium hydroxid solution. Allow the flask to stand in a warm place for about forty-eight hours. At the end of this time examine the contents of the beaker. What is the precipitate at the bottom? How has it been formed?

Remove the tube from the flask, and convert the flask into a distilling apparatus, using the Liebig

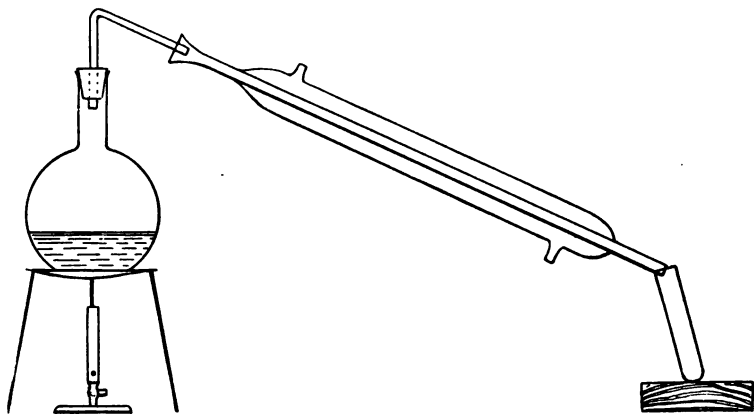


FIG. 2.—Apparatus for distillation.

condenser and adapter as shown in Fig. 2. Distill off about 20 c.c. of the yeast mixture, using an asbestos board to protect the bottom of the flask. Divide the distillate into three portions and use for the following tests :

a. Add to the first portion a few drops of a concentrated solution of iodine in potassium iodide, and warm gently. Add to the mixture sufficient potas-

sium hydroxid solution to decolorize the iodine. Warm again, and allow to cool.

Note the smell of the mixture and the formation of a light-yellow precipitate. Allow the precipitate to settle, and remove a portion with a pipette. Transfer to a microscopic slide, and examine with the microscope. Note the appearance of either hexagonal plates or of six-pointed star-shaped crystals. What are these crystals?

b. Heat the second portion with a drop of sulfuric acid, and add a few drops of a dilute solution of potassium dichromate. Note the change in color of the red of the dichromate solution to green. Note the odor of the solution. To what is it due? What is the reaction which has taken place?

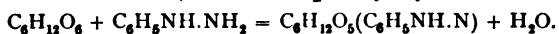
c. To the third portion of the distillate add a few drops of sulfuric acid and an equal quantity of acetic acid. Heat the mixture to boiling, and note the odor of the vapor which is given off. What is it? How has it been formed?

Write the reaction which has taken place when the glucose has been fermented with yeast.

Of all the tests for glucose which may be considered characteristic, that with phenylhydrazin is by far the best.

It differs from the reduction tests in giving a definite chemical compound as the result of the reaction. This compound is characterized by well-defined properties, such as melting-point and crystalline peculiarities. The test is performed by treating the solution containing the glucose with an excess of phenylhydrazin at 100° C. The re-

action proceeds in two stages. In the first a soluble product is formed, which is phenylhydrazon :



This substance, which is not isolated, reacts with another 2 molecules of phenylhydrazin, giving phenylglucosazon, anilin, ammonia, and water. In this reaction the H.C.OH group next to the C:O.H group in the glucose is oxidized to C:O. At the same time 1 molecule of phenylhydrazin is reduced to anilin and ammonia. The third molecule of phenylhydrazin reacts with this group to give a second



and water. The reaction is as follows :



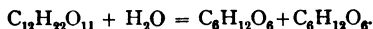
Phenylglucosazon is a brilliant-yellow solid, which is almost insoluble in water. It crystallizes in beautiful acicular crystals, which may appear alone or in the form of sheafs or bundles, which are exceedingly characteristic. It is soluble in hot alcohol, and crystallizes from that solvent on cooling in the form of needles which melt at 204° C. In the urine the only substance which will give a similar crystalline precipitate is glycuronic acid. The yellow compound formed with this substance and phenylhydrazin melts at 104° C. Further, if the glycuronic acid be heated with phenylhydrazin at 100° C. for an hour, the compound is decomposed. This does not happen with phenylglucosazon, which is perfectly stable at that temperature.

The test is performed in the following way :

Into a test-tube are placed about 1 centimeter of solid phenylhydrazin hydrochlorid and an equal bulk of dry sodium acetate. On this are poured 10 c.c. of the solution containing glucose. The test-tube and the contents are placed in a water-bath or beaker of water heated to boiling, and allowed to remain there for an hour, the water being kept at 100° during this time. If glucose be present in the solution, the characteristic yellow precipitate will be found, especially if the tube be cooled slowly. A small amount of the precipitate is withdrawn with a pipette, and transferred to a slide, and examined with the microscope.

Levulose.—Levulose is of such rare occurrence in the animal organism that its reactions will not be taken up here. It is distinguished from glucose, among other things, by its turning the plane of polarized light to the left.

Galactose is formed by the inversion of lactose with acids. When lactose is boiled for some time with dilute acids it takes up a molecule of water and yields galactose and glucose :



Saccharose.—Cane-sugar is not found in the animal organism, but it plays such an important part as a food-stuff that it is a prominent factor in all questions of metabolism.

1. Take 0.5 gram of sugar in a test-tube. Pour on it a few c.c. of concentrated sulfuric acid. If the reaction does not start at once, warm the tube.

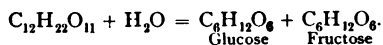
Note the formation of carbon, by the extraction of water from the carbohydrate molecule. Does glucose act in this way with sulfuric acid?

2. Make a 2 per cent. solution of saccharose in water, and use for the following tests: Heat 5 c.c. with an equal volume of potassium hydroxid solution. Does browning take place?

3. Apply Fehling's test as was directed in the section on Glucose. Does saccharose reduce Fehling's solution?

4. Test a solution of saccharose with Molisch's test.

5. Saccharose, in common with other disaccharids and polysaccharids, has the property when heated with acids and water of taking up a molecule of water, and of being converted into monosaccharids. This also occurs when, instead of water, certain salts, such as copper sulfate, aluminium chlorid, etc., are used. In the case of saccharose the two monosaccharids formed are glucose and fructose. They are formed in equivalent quantities, as the following reaction indicates:



As the levorotatory power of levulose is greater than that of glucose, the mixture of the two substances, which is called "invert sugar," is levorotatory.

The action of acids and salts on saccharose and other complex saccharids is due to hydrogen ions furnished by these salts and acids. This reaction is therefore used to detect hydrogen ions in solution,

and the rapidity of inversion is employed as a means of estimating the amount of these ions in a solution.

Certain ferments, also, have the power of converting cane-sugar into simpler carbohydrates, and for this reason a dilute solution of saccharose will ferment if a sufficient quantity of yeast be present. This is due to a specific ferment of yeast, the invertin, which first changes the saccharose to glucose and fructose, and these compounds subsequently ferment with yeast.

Take about 50 c.c. of saccharose solution in a small flask, add to it 0.5 c.c. of strong hydrochloric acid, and close the mouth of the flask with a rubber stopper. Heat the flask in a water-bath for about fifteen minutes. At the end of this time remove the flask and neutralize the acid with dilute potassium hydroxid.

Test the mixture for glucose with Fehling's solution. Is a reducing sugar present?

Make a comparison table of the reactions of saccharose and glucose.

Lactose.—The elementary reactions for lactose are not characteristic.

Try the reactions with potassium hydroxid, Fehling's solution, and with Molisch's reagent. Compare the results obtained with glucose and saccharose.

Starch.—Starch is found in all plants, and makes up the greater proportion of the carbohydrates used as food-material. In plants it is found organized in the form of granules which

have a concentric structure, which is more or less characteristic for each plant. The granules react with polarized light, producing alternate dark and light areas, in the form of a cross, the center of which is at the hilum or nucleus of the granule (Fig. 3).

Starch is, under ordinary conditions, perfectly insoluble in water. When heated with water to



FIG. 3.—Potato starch.

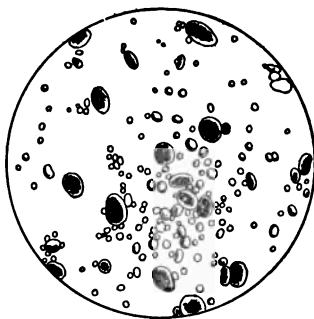


FIG. 4.—Wheat starch.

boiling, the structure of the granule is destroyed, and the starch is converted into a pasty mass which is opalescent in more dilute solution. This so-called soluble starch is obtained more completely when dry starch is heated with water under pressure.

When dry starch is heated to 150° – 160° C. it turns yellowish brown, and is converted into a soluble compound called dextrin, which is the basis of ordinary mucilage.

As with the disaccharids, starch when heated in water with dilute acids is inverted, and glucose is

formed. All the glucose of commerce is obtained in this way.

When starch is treated with saliva or the succus pancreaticus it is also converted into dextrins or into the sugars maltose and isomaltose.

The most characteristic reaction of starch is its behavior with solutions of iodine. With this reagent starch gives a beautiful blue color in the cold, which disappears on heating the solution and reappears on cooling. Conversely, this test serves to detect extremely small traces of free iodine in solutions containing it.

1. Examine a minute quantity of starch under the microscope by suspending it in a small quantity of water, and covering the preparation with a cover-slip. Examine the following starches; and draw two granules of each:

Potato-starch, corn-starch, wheat-starch, and arrowroot.

Examine one of the samples with the polarizing attachment, and rotate the analyzing prism. Note the change in appearance with change in position of the analyzer.

Add a drop of a very dilute solution of iodine to a microscopic preparation with a glass rod. Note the change to blue in the color of the granule.

2. Suspend 1 gram of starch in 200 c.c. of water and add the mixture to 100 c.c. of boiling water in a beaker. Note the change in the appearance of the mixture. Examine a drop with the microscope. Note the disappearance of the organized granule. Heat the mixture in the beaker to boil-

ing for some minutes and use for the following tests :

3. Test the reducing power of the starch solution with Fehling's solution. Does it reduce? Take 10 c.c. of the starch solution, dilute with 40 c.c. of water, and add 0.5 c.c. of sulfuric acid. Boil for ten minutes in a small flask.

Cool, and neutralize exactly with potassium hydroxid solution. Test the reducing power of the resulting mixture with Fehling's solution. Does it reduce? What has happened?

4. Try the reaction with Molisch's test, and with potassium hydroxid.

5. Heat about a gram of dry starch in a dry test-tube. Keep the starch in motion by shaking the test-tube. Note the change in color of the starch. Cool the tube, and add water. Has a soluble compound been formed? Filter off some of the solution and add iodine solution to the filtrate. Note the reaction with the iodine solution. What is the substance?

Glycogen.—This important compound is formed in the liver, and would appear to be the intermediate compound between the carbohydrates taken in as food and the final products of oxidation, when these are used up by the oxygen of the oxyhemoglobin. It is also formed to some extent from protein substances, and serves as a reserve store of food-material in the liver. In its formation from protein substances a chemical change takes place in which the protein molecule is broken up, yielding certain nitrogen free compounds, the

nature of which is not known. Glycogen is found to a large extent in certain mollusks, and in lower plant organisms, among which are the fungi.

It is conveniently prepared either from perfectly fresh liver or from oysters. The tissue from which it is to be prepared is minced up finely, and thrown into boiling water made slightly acid with acetic acid. The mixture is strained through muslin, and the filtrate concentrated on the water-bath. The protein substances, especially the gelatins, are removed by the cautious addition of potassium mercuric iodid solution and dilute hydrochloric acid.

When a precipitate ceases to be formed the mixture is filtered, and the filtrate tested with the mercuric reagent, in order to see that the solution is free from nitrogenous material in solution. The glycogen is precipitated by the addition of 2 volumes of strong alcohol to the solution, and is allowed to settle. The mother-liquor is poured off, and the precipitate is covered with absolute alcohol. After standing some time the alcohol is removed and the residue washed with a little ether, and finally placed on a filter-paper and dried in a desiccator over sulfuric acid. It is a white powder, which is excessively hygroscopic, and for this reason is best preserved in small sealed glass tubes.

1. Mix a small quantity of glycogen with a little water. Note the opalescence of the solution. Divide the liquid into two parts. To one portion add a drop of acid. Note the clearing of the liquid. Add a drop of alkali to the other. Note that it also clears.

2. To a solution of glycogen add a drop of dilute iodine solution. Observe the reddish-brown color of the liquid. Heat the tube. Note the disappearance of the color. It reappears on cooling. Compare with the behavior of starch.

3. Test a solution of glycogen with Fehling's solution. Does it reduce? Heat the glycogen solution with a drop of acid. Test the reducing power, after neutralizing the solution. What has been formed? Compare with the behavior of starch.

Dextrin is formed by heating starch to 150° – 160° C., and also importantly by the action of saliva on starch. It also occurs in several prepared foods, which are made by heating starchy materials to a definite temperature. The dextrans, as found, are scarcely to be considered as chemical individuals. Three forms of dextrans are recognized: erythro-dextrin, achroödextrin, and maltodextrin. The first is so called because of the red color which its solutions give with iodine. The second and third yield no distinct coloration with that reagent. Maltodextrin, is formed by the action of malt diastase on starch. The dextrans are the intermediate compounds between the starches and the monosaccharides. All of the dextrans reduce Fehling's solution slightly.

Cellulose forms the framework of practically all plants. It is not found in the higher animals, and its reported occurrence in pathological conditions, in the fibrous growths of tubercular tissue, is still a matter of doubt. It is difficultly soluble in most

solvents. Schweitzer's reagent (an ammoniacal solution of cupric oxid) dissolves it, and it is precipitated unchanged from this solution by water, alcohol, and acids.

Its chief value in the animal economy is to furnish an insoluble bulky material which will encourage peristalsis. One of the most important uses to which cellulose is put in physiological chemistry is the preparation of the parchment paper used in dialysis. Parchment paper is made by immersing ordinary paper in 60 per cent. sulfuric acid for a few seconds, and washing immediately with water till the paper no longer exhibits an acid reaction. Paper so prepared is much tougher than ordinary paper, and does not disintegrate in water. Made up in the form of tubes, it is used for dialyzing, as it allows the salts which are contained in a solution to pass through the very fine interstices of the paper, while substances of high molecular weight, such as the albumins, are prevented from passing through. In this way a solution containing salts and albumins may be freed from the former.

The Polarimeter.—Many of the methods for estimating the carbohydrates quantitatively are based on polarimetric methods.

Each carbohydrate, roughly speaking, has the power of rotating the plane of polarized light either to the right or to the left to a definite extent. If a known quantity of the compound be dissolved in a given weight of water, and be introduced into a tube of definite length, the column of liquid so produced will have the property of rotating the plane

of polarized light through a definite number of degrees. Conversely, if it be found that a column of liquid of the same dimensions be capable of turning the plane of light through a given number of

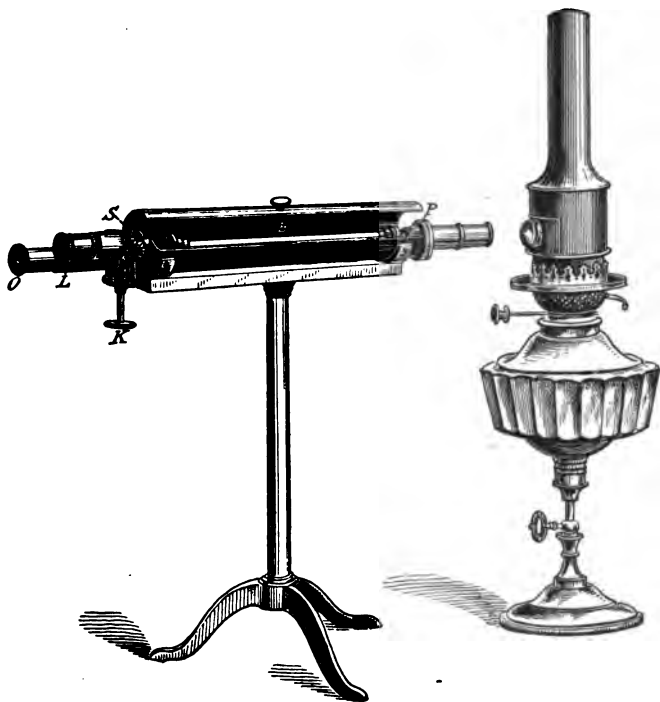


FIG. 5.—The Schmidt and Haensch polariscope.

degrees, one should be able to calculate the amount of carbohydrate in solution. It is on this principle that many of the methods for the estimation of cane-sugar and of glucose are based.

Although not the most accurate, the most satis-

factory polarimeter in use in physiological chemistry, and especially for the estimation of glucose in urine, is the saccharimeter of Schmidt and Haensch. This instrument is so constructed that with a 200 mm. tube the readings on the scale are given as glucose in per cent., and fractions of a per cent.

A description of these instruments will be found in any elementary book on physics.

THE FATS.

The fats are found in nearly all the tissues of the body, and are also an important element in the food. Chemically they are the salts, or esters, of oleic, palmitic, and stearic acids, in which the alcohol is glycerol. As this alcohol is triacid and the acids are monobasic, it follows that to form neutral salts 1 molecule of the alcohol is combined with 3 molecules of the acids. As a consequence the fats are often spoken of as triglycerids.

The principal fats and their formulæ are :

Olein,	glyceryl trioleate,	$C_3H_5(C_{17}H_{33}COO)_3$;
Palmitin,	glyceryl tripalmitate,	$C_3H_5(C_{16}H_{31}COO)_3$;
Stearin,	glyceryl tristearate,	$C_3H_5(C_{17}H_{35}COO)_3$.

While stearin and palmitin when pure are solid at ordinary temperatures, the compound of oleic acid with glycerol is a liquid. At the temperature of the body—37° C.—the mixture of glycerids is liquid, but solidifies when cooled to room temperature. In milk and butter the glycerol is combined with lower fatty acids—caproïc, caprylic, and butyric acids. These acids are volatile with steam, while

the higher fatty acids do not come over on distillation with water. It is on this property that the recognition of artificial butter is based, as margarin or "oleomargarin," which is made of animal fats, does not contain the volatile fatty acids which are found in butter.

The higher fatty acids are insoluble in water, sparingly soluble in alcohol, and easily soluble in ether and in chloroform.

The sodium and potassium salts of these acids are known as the "soaps." When a mineral acid is added to a solution of these soaps the compound is decomposed, and the acid itself is precipitated as a white, tallow-like substance which crystallizes from ether or alcohol in characteristic forms.

On heating the fats with alkalies or with superheated steam they are decomposed, yielding in the former case the alkaline salts of the fatty acids and glycerol, and in the latter case the acids themselves and glycerol. This process is called saponification, and in this way the soaps are made.

The fats themselves are insoluble in water, but under certain conditions, viz., in the presence of small quantities of soaps, they can be finely divided or emulsified. This takes place in the intestine, and in this state the fats are absorbed by the membrane of the duodenum and jejunum, and the process can be imitated in the laboratory. All fats as occurring in nature contain traces of free fatty acids, and these latter in the presence of the alkaline fluids of the bowel are converted into soaps. Through the peristaltic action of the bowel, and

in the presence of these small quantities of soaps, the fats are subdivided, and pass through the bowel-wall and are absorbed.

Saponification; Preparation of Fatty Acids.

—Take about 5 grams of mutton- or beef-fat in a small flask, add 25 c.c. of strong alcohol, and 5 c.c. of a concentrated solution of potassium hydroxid. Fit the flask with a cork, through which passes a piece of glass tubing about 100 cm. long (Fig. 6). Heat the flask gently over an asbestos board for fifteen minutes. Take care that the vapors of the alcohol are condensed by the air-cooling of the long tube, and do not escape. At the end of fifteen minutes dilute the mixture with 100 c.c. of water, and carefully add dilute sulfuric acid till the contents of the flask react acid.

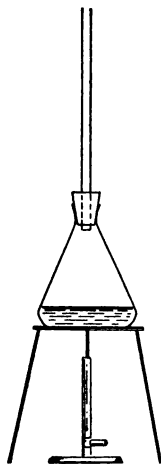


FIG. 6.

Note the formation of solid fatty acids. Filter these off through a small filter, and wash with water. Dissolve the acids in a small quantity of alcohol, using heat. Pour off a few drops into a watch-glass, and allow the solvent to evaporate. Examine the residue left, with the microscope. Sketch some of the crystals formed.

Test the reaction of the alcoholic solution of fatty acids with a few drops of phenolphthalein, made slightly red with a drop of very dilute alkali.

Volatile Fatty Acids.—Saponify 5 grams of

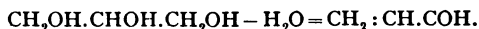
butter-fat, using the same apparatus as was employed in the case of the beef-fat. Make the mixture acid with dilute sulfuric acid as before, and distil the contents of the flask, arranging a distilling apparatus as shown in Fig. 2.

Put into the distilling apparatus a few pieces of porous tile or pumice-stone, to prevent the mixture bumping.

Collect about 10 c.c. of the distillate. Note the odor and taste of the distillate. Test its reaction with litmus-paper. What are the acids which have come over?

Write the equations of the reactions occurring in the process.

Formation of Acrolein (*Acrylic Aldehyde*) from Fats.—Take a small quantity of tallow, and mix with an equal quantity of acid potassium sulfate in a mortar. Transfer about 2 grams of the mixture to a test-tube, and heat the contents of the test-tube over a flame. (This operation should be performed in the draught cupboards.) Note the odor of the acrylic aldehyd formed by the dehydration of the glycerol :



Emulsification.—Take a small quantity of olive oil in a test-tube. Shake with 10 c.c. of water. Observe that the oil separates almost immediately on ceasing to shake the tube. Add 1 c.c. of a dilute solution of sodium carbonate. Shake again. Note that the oil does not settle readily, but that a certain amount remains in the aqueous layer in a

very finely divided state. Allow the tube to stand for an hour. Examine at the end of this time. Describe the appearance.

THE PROTEIN SUBSTANCES.

The proteins are the most important series of substances dealt with in physiological chemistry. They make up a large part of the food which is used by animals, and form the greater part of the solid material of the body. They are also characterized by containing an amount of nitrogen which averages about 16 per cent.

An average of analyses of protein substances gives the following numbers in percentages :

Carbon	52.0
Hydrogen	7.0
Nitrogen	16.0
Oxygen	23.0
Sulphur	2.0

In many of the proteins other elements enter, such as iron and phosphorus. A large percentage of the proteins are insoluble in water. Those which are soluble do not form a true solution, as, for example, sugar does, but exist, as do the soaps, in a colloidal or jelly-like condition. For this reason the molecular weight of these compounds has not been ascertained, and hence no formula can be given to them. Those determinations which have been made lead to the assumption that the molecular weight of egg-albumin is about 15,000, and that of vitellin 5513 with the formula

$C_{292}H_{481}N_9S_2O_{83}$. These formulæ have as yet no real scientific foundation. The molecular weight of these substances is, however, undoubtedly high. The proteins as a whole are uncrystallizable. Recently a number of plant and animal albumins have been made to crystallize in microscopic crystals, but in the majority of cases this occurs only with the greatest difficulty.

In contradistinction to the crystalloids, the proteins are not dialyzable, and this property is used to separate them from substances, like the inorganic salts, which dialyze readily.

Aqueous solutions of protein substances have the property of being precipitated when their solutions are saturated with easily soluble salts, such as the sulfates of ammonium and magnesium, and the chlorids of sodium and iron.

Many of the proteins react differently with the salt employed, and hence this property is in common use to separate mixtures of these substances. All the members of this series are insoluble in strong alcohol, and may be precipitated out of solutions containing them by it. On long-continued treatment with alcohol the proteins are modified, so that they do not redissolve in the solvent from which they were precipitated. When this occurs the phenomenon is called coagulation, in contradistinction to precipitation, where on the addition of the original solvent the substances are redissolved.

One of the most characteristic reactions of many of the proteins is the change which they undergo

when their solutions are heated. This coagulation may occur either in neutral, acid, or alkaline solution, according to the protein with which one has to deal. In neutral or alkaline solution the coagulation is, as a rule, only partial. In acid media the protein may be entirely thrown out of solution under favorable circumstances. When the acid is employed in too great or too small a quantity the coagulation may here be also partial.

The following is the classification of protein substances according to Neumeister:

The Albumins.—1. The true albumins. The members of the following groups are differentiated by their coagulation-temperatures, their specific indices of refraction, and their behavior toward certain reagents.

a. The albumins. Serum-albumin, egg-albumin, lactalbumin, and plant-albumins.

b. The globulins. Fibrinogen (metaglobulin), serum-globulin (paraglobulin), fibrinoglobulin (from the digestion of fibrin), plant-globulins, and myosin.

c. Vitellins. Phytovittellin and crystallin.

2. Albumins formed from true albumins by fermentative action. Fibrin, formed from metaglobulin by the action of the fibrin-ferment.

3. Artificially changed albumins.

a. Albuminates, formed by the action of alkalies or acids on albumins.

b. Coagulated albumins.

The Proteids.—Compounds formed from the union of an albumin with some other substance.

a. Nucleo-albumins. Compounds of albumins with nucleius. Casein.

b. Glycoproteids. Compounds of albumins with carbohydrates. Mucin, mucoid, and hyalogen.

c. Hemoglobins. Compounds of albumins with coloring-matters containing iron. Hemoglobin, oxyhemoglobin, methemoglobin, carbon-monoxid hemoglobin, etc.

d. Nucleius. Compounds of albumins with phosphoric acid or nucleic acid.

The Albuminoids.—*a.* The keratins (found in hair, epidermis, nails, etc.).

b. The elastins (found in elastic tissue, the intima of the arteries).

c. Collagen (found in the organic part of bones; is the principal constituent of gelatin).

Besides those compounds which are classified above, are the albumoses and peptones formed by the digestion of protein substances. These form a class entirely separate from the above. They will be classified in the section on Digestion.

Egg-albumin.—To prepare the solution for use in the following experiments take 20 c.c. of egg-albumin in a flask of 400 c.c. capacity, and add 150 c.c. of water. Shake the mixture vigorously and filter through a folded filter. The residue on the filter consists of the membranous network in which the albumin is enclosed in the egg.

1. Test the reaction with litmus-paper.

2. Heat 5 c.c. of the solution to boiling in a test-tube. Note that the solution becomes opalescent, but coagulation does not take place. Add a drop

of acetic acid. Note the immediate separation of the albumin.

3. Heat 5 c.c. of the solution with 2.5 c.c. of sodium hydroxid solution. Alkali albuminate is formed, which is soluble. The albumin does not coagulate. Add dilute acetic acid drop by drop, and note the commencing coagulation of the albumin when the alkali is neutralized and the solution becomes faintly acid.

4. To 5 c.c. of the solution add mercuric chlorid solution. A heavy precipitate forms, insoluble in excess of the reagent. Add a concentrated solution of sodium chlorid. The precipitate dissolves.

5. To 5 c.c. of the solution add nitric acid till a precipitate forms. Then add an equal volume of alcohol. Note the persistence of the precipitate.

6. The following reactions are to a greater or less extent characteristic of all proteids. Dilute the egg-albumin solution used in the foregoing tests with $2\frac{1}{2}$ times its volume of water.

a. The Biuret Test.—Mix equal parts of albumin solution and potassium hydroxid. Heat gently and add a few drops of a 2 per cent. solution of copper sulphate. Allow the copper solution to flow down the side of the test-tube. Note the formation of a pink or violet color.

b. Xanthoproteic Reaction.—Add an equal part of strong nitric acid to the albumin solution. Boil. A yellow solution is formed. Place about 1 c.c. of the solution in a porcelain dish, and add strong potassium hydroxid till the mixture is strongly

alkaline. Note the change from deep yellow to orange.

c. Millon's Test.—To 5 c.c. of albumin solution add 0.5 c.c. of Millon's reagent. A white precipitate forms. Boil, and note the change in the color to a deep red.

d. Adamkiewicz' Reaction.—To 2 c.c. of albumin solution add 5 c.c. of glacial acetic acid. Warm, and after heating cool the solution with tap-water. Then add slowly by means of a pipette 5 c.c. of concentrated sulphuric acid, so that the two liquids do not mix. A purplish-violet ring forms at the point of contact.

The Coagulation-temperature of Albumins.—This test is performed in a series of test-tubes, so that all the tubes may be observed under identical conditions.

Take 5 test-tubes, and label them carefully, making a note of the mixture which is put in each.

In I. put 5 c.c. of undiluted egg-albumin.

In II., III., IV., and V., use the diluted solution previously employed for the precipitation-tests; 5 c.c. are placed in each.

To II. add 1 c.c. of 10 per cent. sodium chlorid.

To III. add 4 drops (0.2 c.c.) of the same solution.

To IV. add 2 drops of glacial acetic acid.

To V. add 1 c.c. of a 1 per cent. solution of glacial acetic acid.

Place the tubes together with a thermometer in a beaker containing water, and raise the temperature of the water gradually, noting the temperature at which change occurs in any of the tubes. Raise

the temperature of the water in the beaker to boiling. Note any change that occurs after the temperature has reached 100° C.

After the temperature has been maintained at 100° C., for five minutes add to tubes II. and III. a couple of drops of dilute acetic acid. Note the change which occurs.

To tubes IV. and V. add 1 c.c. of sodium-chlorid solution. Does a change take place? What are the conclusions which can be drawn from these experiments?

Under ordinary circumstances, when urine is tested for albumin, the amount of sodium chlorid present in the urine is sufficient to cause coagulation when the acid is added.

Acid Albumin.—Take 5 c.c. of the dilute albumin solution and add a few drops of hydrochloric acid. Boil the solution. Precipitation does not take place. Acid albumin is formed, which is soluble. Cool and neutralize the acid with potassium hydroxid. Note the change in appearance on the slight excess of acid. Use litmus-paper to find the neutral point.

Precipitation of Protein Substances by Saturation with Easily Soluble Salts.—This method is one of great importance as a means of separating proteins. In some cases it is the only means of separating substances such as serum-globulin and serum-albumin, which are closely allied to one another, and of which the greater part of the reactions are identical.

Heat a water-bath to 35° C. Place in a test-tube

10 c.c. of a dilute albumin solution, and add powdered ammonium sulfate till the crystals are no longer dissolved. Note the appearance after some time of a precipitate. This contains all the globulin and albumin found in ordinary egg-albumin. Filter off the precipitate and test the filtrate with the biuret test. Is a protein substance present in the filtrate?

Treat the precipitate on the filter with cold water. Test the filtrate with acetic acid and heat. Note the ready solubility of the precipitate in water.

Prepare a similar tube, saturating the solution with crystalline magnesium sulfate. Heat at 35° C. for the same length of time. Note the formation of a very slight precipitate of egg-globulin contained in the egg-albumin. Magnesium sulfate precipitates globulins, but does not precipitate albumins. Filter the solution, and apply the acetic acid and heat test. Note the copious precipitate of albumin which has not been precipitated by the magnesium sulfate.

THE BLOOD.

The blood as existing in the body may be classified into arterial and venous. These two kinds are distinguished by their content of oxygen and of carbon dioxid. They are also distinguished by their color, which is due to the blood-pigment being in different stages of oxidation. In arterial blood the pigment consists for the most part of oxyhemoglobin, characterized when in sufficient quantity by its bright-red color and its spectrum.

Venous blood has a darker hue, and the pigment has lost a part of the loosely combined oxygen present in oxyhemoglobin and has been converted into hemoglobin. This also has a characteristic spectrum.

The blood consists, roughly, of two parts, the corpuscles, or organized elements of the blood, and the plasma, in which the corpuscles float.

An average of human blood places the blood-corpuscles at 48 per cent. by weight, and the serum therefore at 52 per cent.

The blood-corpuscles contain a large amount of iron-containing protein substance. This is oxyhemoglobin, and forms about 40.5 per cent. by weight of the corpuscles.

The blood-plasma is essentially a solution of protein substances, in which also are dissolved some salts. It contains 8.9 per cent. of solid matter, of which 6.9 per cent. is protein in character; 0.84 per cent. of this latter is made up of inorganic salts. The inorganic portion of the serum is chiefly sodium chlorid, although salts of potassium, calcium, and magnesium are also present.

The reaction of the blood is alkaline, due to the carbonates of the alkaline metals. Phosphoric acid is also present, but is combined with the proteins into a complex molecule. The protein substances in the blood-plasma are metaglobulin (fibrinogen), paraglobulin (serum-globulin), and serum-albumin. Metaglobulin, the first mentioned of the proteids, is the most important, as it is the precursor of the fibrin formed in the clotting of the blood.

The corpuscles are of two kinds, red and white. The red blood-corpuscles consist of the hemoglobin and stroma. The stroma contains the same chemical substances as are found in other protoplasm—globulins, lecithin, protagon, and cholesterin. The amount of water contained in the red blood-corpuscles is about 57.7 per cent. The chemical constitution of the white corpuscles or leukocytes is not known, owing to the difficulty of their isolation. It has been assumed that they do not differ materially in composition from the corpuscles found in the lymph-glands. These contain, besides globulins, lecithin, protagon, and a nucleo-albumin not precipitated by saturated magnesium sulfate solution, called nucleohiston. This substance is acid in character, and contains a relatively large amount of phosphoric acid—4.99 per cent. Glycogen is also found to a small amount in the leukocytes.

The hemoglobins are immensely important, and their relation to one another should be carefully followed out.

The blood-pigments are: 1. Oxyhemoglobin; 2. Hemoglobin; 3. Reduced hemoglobin; 4. Methemoglobin.

Those formed by the combination of chemical reagents with hemoglobin are: 1. Carbon-monoxid hemoglobin; 2. Hydrogen-sulfid hemoglobin; 3. Cyanhemoglobin (hydrocyanic acid).

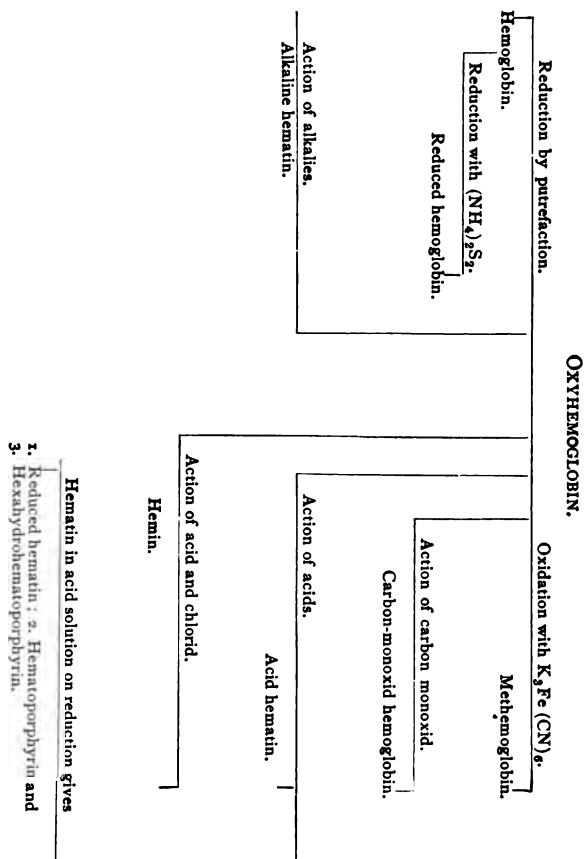
Hemoglobin altered by chemical reagents.

Action of acids—acid hematin. On reduction gives a reduced hematin, oxygen-hematoporphyrin, carbon-hexahydrohematoporphyrin.

Action of alkalies—alkaline hematin.

Action of acids with a chlorid—hemin.

The relation of the different pigments to one another will be seen in the following diagram.



The blood as existing in the blood-vessels is a fluid of a specific gravity of about 1060. On flow-

ing from the blood-vessels and being allowed to stand for some minutes it suddenly becomes solid. The alkaline reaction diminishes and a slight evolution of heat takes place. This phenomenon is known as *coagulation*. Among the many theories which have been advanced to account for this sudden change, part of two may be used. In the first, the process is supposed to be caused by a change taking place in a constituent of the blood-plasma. This substance is fibrinogen. In the second the change is said to be due to a breaking down of the leukocytes. A third factor in coagulation is the presence of a certain amount of calcium salts. All of these may be taken into consideration when seeking for a cause of coagulation.

If fibrinogen be prepared in a pure condition, and dissolved in a slightly alkaline solution of sodium chlorid, on the addition of a small amount of a solution of a calcium salt a typical coagulation takes place. If, on the other hand, a fresh blood be received in a solution of potassium oxalate sufficient to convert all the calcium salts into insoluble calcium oxalate, the fluid loses its power of spontaneous coagulation. The clotting in blood is in part due to a ferment, called the fibrin-ferment or prothrombin, and which belongs to the class of enzymes. It may be prepared from a solution containing it by precipitation with alcohol, in which it is insoluble. If a solution of this substance be injected intravenously, death speedily takes place from multiple thromboses.

That the disintegration of the leukocytes is also a factor in the coagulation of blood may be shown by allowing blood to flow from an artery into a vessel whose walls are smeared with vaselin. Blood received in this way, and which is protected from the air by being carefully covered, will not coagulate for a much longer time than blood which has been allowed to collect in an ordinary open vessel. In the former case the white corpuscles retain their form, as will be seen on microscopic examination.

The leukocytes themselves contain a ferment—nucleohiston—which has the property of causing coagulation. This ferment is peculiar in that when used in larger quantities it has the property of not only not causing coagulation, but of actually inhibiting it.

Chemical Reactions of Blood.—Take a sample of blood, and dilute with 10 times its volume of water, and use for the following tests.

1. Reaction to litmus. Saturate a plate made of plaster of paris, by making a cream of the calcium sulfate with water and pouring on a clean glass plate, and allowing to set. Pour on the plate some litmus solution, which should be neutral or very slightly acid. Touch the plate with a drop of fresh blood, wash off the excess of blood, and examine the spot. This test may be performed with litmus-paper, but the reaction is not so well marked.

2. The catalytic action of blood. To 5 c.c. of hydrogen peroxid add 1 c.c. of blood. Note the

foaming of the mixture, due to the disengagement of oxygen gas from the peroxid.

3. Make a fresh alcoholic solution of gum guaiac by dissolving a minute fragment of the gum in 2 c.c. of alcohol. Introduce a few drops of this solution into the diluted blood. A precipitation of the resin takes place. Add a few cubic centimeters of commercial turpentine. Note the blue ring formed at the junction of the two fluids. Shake, and note the blue color of the mixture, and the blue froth.

The Spectroscopic Examination of Blood.—
Oxyhemoglobin.—Examine the dilute solution of blood in a test-tube with a hand spectroscope. Note the appearance of the bands. Dilute with water, and note the appearance of the bands in the diluted blood.

Hemoglobin.—Examine the spectrum of hemoglobin, made by allowing blood to undergo putrefaction in the absence of air. Note the color of the solution and the appearance of the bands.

Reduced Hemoglobin.—Take the dilute solution of blood, and add a few drops of yellow ammonium sulphid solution. Warm gently to about 40° C. Note the change in color to a purplish tint. Examine the spectrum of this solution. It has the characteristics of reduced hemoglobin. Reduction of the oxyhemoglobin may also be accomplished by other reducing agents, of which Stokes' solution is the best. This is prepared by dissolving 1 part of ferrous sulfate in 10 parts of distilled

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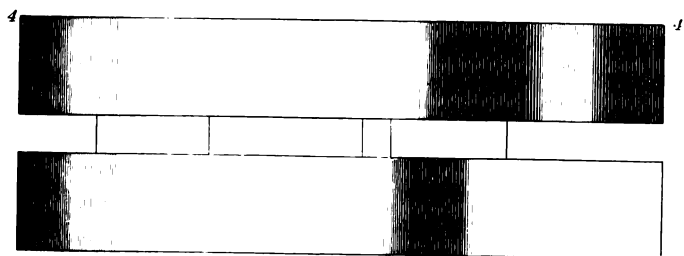
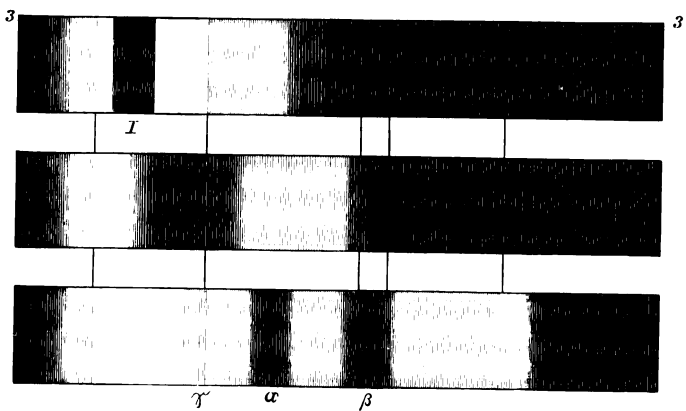
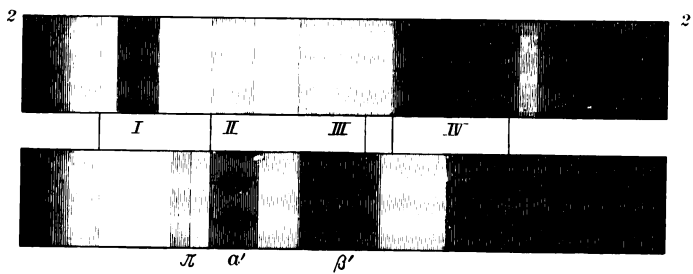
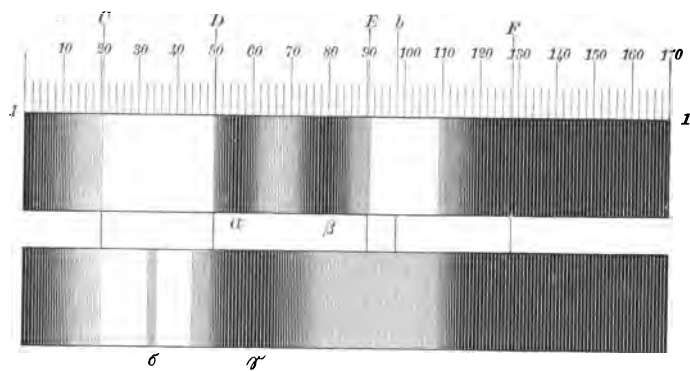
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PLATE I.

Spectra (after Neubauer and Vogel).

1. *a*, Oxyhemoglobin; *b*, hemoglobin free from oxygen.
2. Methemoglobin : *a*, in neutral solution; *b*, in alkaline solution.
3. *a*, Hematin in acid alcoholic solution; *b*, in ammoniacal solution;
reduced hematin.
4. *a*, Urobilin in acid solution; *b*, zinc salt in ammoniacal solution.



water, and adding the resultant mixture to 6 parts of ammonia and filtering.

Carbon-monoxid Hemoglobin.—Attach a piece of rubber tubing to a gas-tap, and provide this with a small piece of glass tube sufficiently long to reach to the bottom of a test-tube. With the aid of this tube pass a slow current of coal-gas through the blood solution. Note the change to a bright cherry red in the color of the blood. Examine the spectrum of this solution. Two bands appear, which are situated very near the bands of oxyhemoglobin. Compare with a similar solution of fresh blood. It will be found that the bands of carbon-monoxid hemoglobin are fainter and less well defined. On careful examination with a spectroscope with a scale attached, it will be seen that the bands are somewhat nearer the violet end of the spectrum than are those of oxyhemoglobin.

Treat the solution of carbon-monoxid hemoglobin with ammonium sulphid. Examine the solution with the spectroscope. Note that the spectrum remains unchanged. Carbon-monoxid hemoglobin is not affected by reducing agents.

To 10 c.c. of carbon-monoxid hemoglobin solution add an equal volume of sodium hydroxid solution. Note the bright appearance of the mixture. Treat a solution of oxyhemoglobin in the same way. Observe the appearance of a dirty-brown precipitate or coloration.

Methemoglobin.—Methemoglobin is an oxidation-product of oxyhemoglobin. It is produced by the action of weak oxidizing agents such as potas-

sium ferricyanid. It is found in the blood in cases of poisoning by strong oxidizing agents, such as potassium chlorate.

To a concentrated solution of blood add a few drops of a freshly made solution of potassium ferricyanid. Allow to stand for some minutes, and note the change in color to a brownish tint. Examine the diluted solution with the spectroscope. The spectrum is especially characteristic. Almost complete absorption takes place in the blue region of the solar spectrum. A narrow band will be found near the position of the narrower oxyhemoglobin band, and a well-marked band occurs toward the red end of the spectrum.

Alkaline Hematin.—Solutions of alkaline hematin are prepared by heating blood-pigment with alkalies. The hemoglobins break up under this treatment, yielding a hematin-pigment and an albumin. When oxyhemoglobin is treated in this way the compound hematin results. When hemoglobin is boiled with alkalies in the absence of air, reduced hematin results.

Heat 10 c.c. of blood solution with 1 c.c. of sodium hydroxid. The color of the solution changes to a brownish green. The spectrum of this solution is not characteristic; complete absorption takes place in a part of the red spectrum. If this solution be reduced with yellow ammonium sulfid or with Stokes' reagent, one obtains the spectrum of reduced hematin or hemochromogen. The spectrum of this compound is scarcely to be distinguished from that of oxyhemo-

globin. The absorption-bands, however, are somewhat nearer the violet end of the spectrum than are those of oxyhemoglobin.

Hemin.—Hemin is of importance because of the bearing which it has on the examination of substances supposed to contain blood. It was formerly supposed to be the hydrochlorid of hematin. It is now known that the chlorin is not present as the acid part of a salt, but is a part of the organic

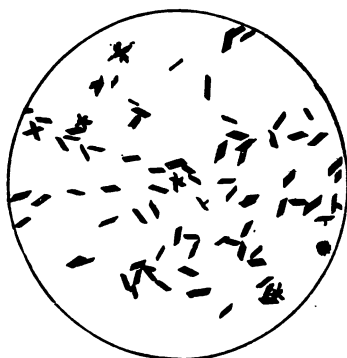


FIG. 7.—Teichmann's hemin crystals.

molecule itself. It has well-defined crystalline characteristics, which enable it to be easily recognized. It is insoluble in ordinary solvents, and crystallizes in shining metallic plates. It is prepared by the action of acetic acid and sodium or ammonium chlorid on ordinary blood-pigment.

The Hemin-test for Blood.—Take a minute quantity of dried blood on a microscopic slide, or, if the blood be in solution, evaporate it, using no

more heat than is absolutely necessary. Add a very small fragment of sodium or ammonium chlorid to the blood, and a drop of acetic acid. Cover with a cover-slip. Heat the slide with a small flame, taking care that the acid is just brought to a boil, but does not vaporize. Allow the preparation to cool, and examine with a microscope, using a medium objective. Note the formation of the characteristic crystals (Fig. 7). The test may also be performed by using a piece of cloth which is blood stained. The stain is cut out of the cloth, and placed on a slide, and treated with the chlorid and acetic acid. The crystals form readily on the fibers of the cloth.

Hematoporphyrin.—This compound is of importance, because it occurs not infrequently by the breaking down of blood-pigment in the body following the use of certain compounds, among which may be mentioned sulfonal. It may be prepared by the reduction and simultaneous elimination of iron from the hemoglobin or hematin molecule. In contradistinction to the other blood-pigments, it contains no iron. The anhydrid may be made from hemoglobin by acting on that substance with concentrated sulfuric acid. The solution has a brownish-black color, and it is this color which leads one to suspect the presence of hematoporphyrin in urines having a black color.

The Blood-plasma.—The blood-plasma is essentially a solution of albumins. Other compounds, such as uric acid, glucose, and inorganic salts, are found in solution, but they occur, at the

most, only in traces. Their presence is relatively unimportant.

By heating blood diluted 1:8 a coagulum is obtained, consisting of fibrin colored brown with altered blood-pigment.

If the coagulum be filtered off, a filtrate is obtained containing a small amount of glucose and inorganic salts.

The protein substances existing in solution, besides the fibrin, are serum-albumin and serum-globulin. These may be separated by means of magnesium or ammonium sulfate. If ammonium sulfate be added to a solution containing the globulins and albumins, on a proper concentration of the ammonium salt a precipitate of albumins occurs, and the globulins are left in solution. If magnesium sulfate be employed, the precipitate consists of the globulins. This globulin precipitate is redissolved on adding water. If the globulin solution so obtained be dialyzed against distilled water, the inorganic salt passes out and the globulin is left behind. As it is insoluble in pure water, it is precipitated, and will be found as a white flocculent substance adhering to the walls of the dialyzing tube. As has been seen, globulin is soluble in dilute salt solution, but is insoluble in distilled water. Albumin, on the other hand, is soluble both in solutions of salts and in distilled water.

The most convenient apparatus for dialysis for physiological purposes is the dialyzing tube. This is made of parchment-paper formed into tubing vary-

ing from 20 to 75 mm. in diameter. The paper is soaked in distilled water, and the solution to be dialyzed is placed in the tube, which is caught up at both ends and fastened by transfixing the ends with glass rods. The tube and its contents are then placed in a vessel of water, and allowed to remain in contact with the water, till, on testing, the inorganic salts are found to have dialyzed out into the water contained in the beaker. It is advantageous

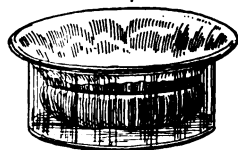
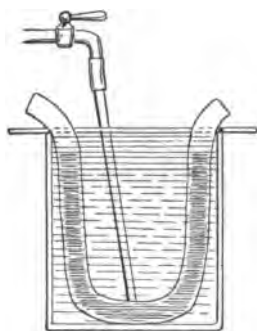


FIG. 8.—Apparatus for dialysis. FIG. 9.—Apparatus for dialysis.

to commence the dialysis by substituting tap-water for the distilled water, which must be frequently changed or made to flow continuously as shown in Fig. 8. At the end of forty-eight hours the flow of tap-water may be stopped, and distilled water introduced into the beaker. Another form of dialyzing apparatus is shown in Fig. 9.

Fibrin.—The fibrin used in these experiments may be made from fresh blood, by whipping it with a bunch of twigs or feathers, and washing the

resulting coagulum from blood with distilled water. The fibrin so prepared may be preserved in glycerin for future use.

1. Allow a few flakes of fibrin to remain in contact with 0.3 per cent. hydrochloric acid for some time. This acid is prepared by diluting 7.5 c.c. of the fuming hydrochloric acid to 1000 c.c. Heat the tube containing the hydrochloric acid and fibrin to 40° C. The fibrin dissolves, and acid albumin is formed. The fibrin preserved in the glycerin will be used later on in the experiments on digestion.

Separation of Serum-albumin and Serum-globulin.—Take 25 c.c. of blood-serum, obtained by whipping fresh blood, and add to it an equal volume of a saturated solution of ammonium sulfate. Note the precipitation of serum-globulin. Filter off, and wash the precipitate with saturated ammonium sulfate solution. Dissolve the precipitate in water, and use for the following tests:

1. Try coagulation by heat and acetic acid.
2. Test the solution with the biuret reaction.
3. Try the xanthoproteic reaction.

The Preparation of Pure Globulin.—To 25 c.c. of serum add powdered magnesium sulfate till the salt ceases to be dissolved. Keep the test-tube containing the solution at 35° C. for some time. Stir the contents of the tube with a glass rod. Filter off the precipitate, and wash it into a dialyzing tube which has been previously soaked in water. Make the contents of the tube up to about 20 c.c. Place the tube in a beaker of water,

and allow a slow current of water to flow into the beaker, and to overflow. Arrange the apparatus as shown in Fig. 8. Care must be taken that the tap-water does not have access to the inside of the dialyzing tube. Allow the dialysis to proceed for some days, and at the end of this time replace the tap-water with distilled water. Allow the dialysis to go on for three days more, and replace the distilled water by fresh at least every day. Open the dialyzing tube over a beaker. The sides of the tube will be found covered with a white film of pure globulin. The globulin is not entirely insoluble in distilled water, and hence a certain amount will be found in the water taken out of the dialyzer.

Serum-albumin, which is found in blood-serum, is in many respects identical with egg-albumin. One of the reactions which serves to distinguish it from egg-albumin is its behavior with ether. When solutions of egg-albumin are shaken with ether the albumin is coagulated. Test a small quantity of serum-albumin by shaking it with ether. Does it coagulate?

The Alkalinity of the Blood.—Quantitative Estimation.—This estimation has come into recent prominence as a clinical feature of examinations of cases of rheumatic and gouty affections in which the alkalinity of the blood has been found to be decreased.

The only special piece of apparatus needed is a pipette made after the model of the Thoma-Zeiss pipette used in hematocytometric estimations. This

pipette is made on a larger scale to contain up to the mark on the capillary tube 0.05 c.c. of blood. The bulb of the pipette contains about 2 to 3 c.c. The solutions required are a $\frac{N}{100}$ solution of tartaric acid,

made by dissolving 0.75 gram of tartaric acid in 1000 c.c. of water. Each cubic centimeter of this solution corresponds to 0.0004 gram of sodium hydroxide, or 0.00053 gram of Na_2CO_3 .

The blood is withdrawn from the finger by means of the pipette till it reaches the mark on the capillary tube. Distilled water is then drawn in till the bulb of the pipette is almost filled. The mixing of the blood solution is effected as in blood-counting. The dilute solution of blood is then expelled into a small beaker, and the pipette washed out with a little distilled water. The solution of

blood is then titrated with the $\frac{N}{100}$ tartaric acid in the usual way, using strips of sensitive litmus-paper to show when the change in the reaction of the blood has taken place. As the amount of blood which has been used is 0.05 c.c., the amount of tartaric acid used must be multiplied by 20 to give the alkalinity of 1 c.c. of blood.

Example.—Took 0.05 c.c. of blood, diluted with water, and titrated with $\frac{N}{100}$ tartaric acid. Used 1.9 c.c. of the acid solution. Hence, $1.9 \times 0.0004 = 0.0076$ NaOH in 0.05 c.c. of blood. Therefore, $0.0076 \times 20 = 0.0156$ = amount of NaOH equivalent to the alkalinity of 1 c.c. of blood.

THE DIGESTIVE FLUIDS, AND THE PRODUCTS OF DIGESTION.

The Saliva.—The saliva as collected from the mouth is a mixture of the secretion of the parotid, submaxillary, and sublingual glands. The amount secreted in the twenty-four hours is estimated at 1500 c.c. It contains about 0.5 per cent. of solid matter, which consists chiefly of serum-albumin, mucin, and the ferment ptyalin, as the organic part; and sodium bicarbonate and potassium sulfocyanid as the inorganic part. The former salt is of interest, as giving the alkaline reaction to the saliva, and also as being transformed into sodium carbonate and carbon dioxid when saliva is allowed to stand.

The potassium sulfocyanid is secreted by the parotid gland alone.

The functions of the saliva are, first, to furnish sufficient water to mix with the solid food-materials, in order to render them fluid—in a state to be acted upon by the gastric and pancreatic juices; secondly, to convert some of the starchy material into soluble compounds, of which dextrans, maltoses, and glucose are the chief.

Saliva for the following tests may be obtained by first rinsing the mouth with water, and chewing a piece of paraffin-wax for a few minutes. The nerves supplying the glands are stimulated, and a copious supply of saliva results. The character of the secretion of the different glands seems to vary, and their action on starch is also connected with

the presence of micro-organisms, so that a perfectly sterile saliva, such as has been obtained by canalizing the ducts under perfectly aseptic conditions, has been found to be without action on starch. With the advent of micro-organisms from the air the fluid regained its diastatic power.

Make a solution of soluble starch by heating 1 gram of starch powder with 100 c.c. of water.

1. To 10 c.c. of the starch solution warmed to 40° C. add 1 c.c. of saliva. Note an almost instant clearing of the mixture. To a few cubic centimeters of the resulting fluid add a drop of iodine solution. Note that the color is brown, or even faintly yellow. Compare the color with that obtained by treating an equal amount of the original starch solution with a drop of iodine solution.

2. Heat a few cubic centimeters of saliva to boiling. Test the power of the solution on cooling to clear a starch solution. Is the starch converted into dextrins and maltoses by the boiled saliva?

3. Treat 1 c.c. of saliva with a drop of hydrochloric acid. Add a drop of dilute ferric chlorid. Note the formation of a red color, due to ferric sulfocyanid.

EXAMINATION OF THE CONTENTS OF THE STOMACH.

The digestive fluid of the stomach is an almost clear liquid having a strong acid reaction. It is obtained either by means of the stomach-tube or through a permanent or temporary fistula. As

obtained from the stomach it is always mixed with saliva.

Unmixed with food materials it is essentially a solution of hydrochloric acid, containing about 0.3 per cent. of that compound, with pepsin, the proteolytic ferment of the stomach, and rennin, the milk-curdling ferment. As might be expected, the fluid always contains a large number of micro-organisms, which, with the exception of the lactic-acid bacillus and certain pathogenic microbes, favor the digestive process. The development of these micro-organisms is, however, inhibited by the amount of acid present in the normal gastric juice. If the amount of the hydrochloric acid be increased above normal, or especially if it be decreased in quantity below that present in health, the growth of the organisms is not only not impeded, but the gastric contents form an excellent culture-medium for them. The secretion of the gastric juice is not continuous, but is dependent on mechanical stimulation, and more particularly on the psychical factor. It is therefore more abundant before taking food, especially if the subject be hungry, and after the completion of a full meal. The degree of acidity of the juice and its digestive activity are also dependent on the quality of the food taken into the stomach. With the ingestion of proteids the acidity increases, while on a carbohydrate diet the acidity is least. The enzymes found in the contents of the stomach are two: *Pepsin*, the proteolytic ferment, and *rennin*, the milk-coagulating ferment.

Pepsin is found in the stomach of all vertebrates,

with the exception of some of the fishes. It is found in the upper digestive tract of children immediately after birth. It has not been isolated in a pure condition, but according to some observers is a substance which does not give the reactions of the albumins.

Pepsin loses its power of proteolysis when solutions containing it are heated to boiling, and a temperature of 55° C. if prolonged is sufficient to inhibit its action if the solution be neutral, and 65° C. if the solution be acid. Dry powder containing the ferment can, however, be heated to 100° C. without losing its properties.

The products of the action of pepsin on proteins like fibrin are the albumoses and peptones. In gastric digestion the albumoses are formed in the larger quantity, while in tryptic or pancreatic digestion the peptones are the principal product formed.

Pepsin of itself is without action on albuminous materials, a definite quantity of an acid, preferably hydrochloric acid, being necessary for its most effective action.

Rennin, the coagulative ferment of rennet, is the second ferment found in the contents of the stomach. It is more particularly to be found in the portion of the stomach membrane excised from the fundus. It is much more susceptible to change on heating than is pepsin. When a solution of the enzyme containing the physiological amount of hydrochloric acid is heated to 37° - 40° C. for forty-eight hours the action of the rennin is destroyed.

Pepsin under these circumstances remains unchanged. Its characteristic property is to cause a coagulation of milk or of a solution of casein containing salts of calcium.

An **artificial digestive fluid** is prepared by dissolving 1 gram of active commercial pepsin in 1000 c.c. of physiological hydrochloric acid containing 0.3 per cent. of the acid. This is roughly made up by adding 8 c.c. of acid of a specific gravity of 1.20 to 1000 c.c. of water.

An active digestive fluid may also be made up by taking the perfectly fresh stomach of a pig, and, after washing with a little water, scraping the interna and transferring to glycerin. This glycerinated solution of pepsin is active and keeps for some time. The solution made from the pepsin powder should be filtered before use.

In order to imitate the gastric contents as occurring in the stomach, it is necessary to add some of the other substances which are found together with the hydrochloric acid and pepsin. These are :

Albumose-peptone Solution.—Dissolve 2 grams of commercial peptone (Witte's—which consists largely of albumoses) in 100 c.c. of water.

Lactic-acid Solution.—Dissolve 0.8 gram of lactic acid in 100 c.c. of water.

Tests for Free Hydrochloric Acid.—**Methyl-violet Test.**—Use a 0.05 per cent. solution of methyl-violet. Note the change in color when a few drops of the solution are added to 5 c.c. of the artificial gastric juice. Repeat the test, using 5 c.c. of the lactic-acid solution.

Repeat after diluting the lactic acid and the artificial gastric juice with 5 times their volume of water.

Influence of the Presence of Albumoses and Peptones on the Methyl-violet Test.—Dilute 10 c.c. of the hydrochloric acid solution with 10 c.c. of the peptone solution. Test with methyl-violet. Has the presence of the peptone and albumose affected the change in color of the indicator?

Congo-red Test.—Test 10 c.c. of the gastric contents with 1 drop of a 0.5 per cent. solution of Congo-red. Note the change in color, especially well marked on gently heating the solution. Try the same reaction, using the solution of lactic acid. Observe the difference in the color-change.

Dimethyl-amido-azobenzene Test.—Test the solution of hydrochloric acid with a drop of an alcoholic solution of dimethyl-amido-azobenzene. Note the change in the color from yellow to red. Compare with the result obtained with the solution of lactic acid.

Test the two solutions with an alcoholic solution of benzopurpurin 6B. Compare the reactions obtained.

Günzburg's Vanillin-phloroglucin Test.—This test has the advantage of not being influenced by the presence of peptones and albuminous substances which are present normally in the gastric juice. It is best performed with a solution of the two reagents which is freshly prepared. The solution changes on standing, and is not so effective in detecting small traces of free mineral acid.

To prepare the solution, mix equal quantities of

phloroglucin and vanillin, as much as will lie on the extreme point of a pen-knife blade, and dissolve in 1 c.c. of alcohol.

Mix about 1 c.c. of the acid pepsin solution with 0.5 c.c. of the reagent, and evaporate the mixture in a porcelain dish, on a water-bath. Note the formation of a bright-red color, which on examination with the microscope will be found to have a microcrystalline appearance.

Repeat the test, using the solution of lactic acid. Note the occurrence of a yellow residue, which may darken to a light brown.

Tests for Lactic Acid.—To 10 c.c. of a 2 per cent. aqueous solution of phenol add 1 drop of a dilute solution of ferric chlorid. A fine purple color results. Add to the solution a few drops of the pepsin-hydrochloric acid. The color will be to some extent discharged.

Repeat the test, using the lactic acid solution. The blue color will be discharged, and will be replaced by a lemon-yellow coloration.

Take two test-tubes of equal size, and place in each of them 10 c.c. of water and a drop of ferric chlorid solution, which should be dilute. Add to one tube 1 c.c. of the hydrochloric-acid-pepsin solution; to the other add an equal quantity of the lactic acid solution. Note the darkening in color, which is especially well marked on looking down the mouth of the tubes. The hydrochloric acid solution does not give any change in color with ferric chlorid.

Isolation of Lactic Acid.—Lactic acid is easily isolated from its aqueous solution by means of

ether. In distilling off the ether the acid remains behind as a non-volatile syrup. This may be dissolved in water, and will give sharper tests with ferric chlorid, or with ferric chlorid and phenol, than when mixed with the other substances usually found in the contents of the stomach. Shake 10 c.c. of a mixture containing it with 5 c.c. of ether in a small test-tube. Decant off the ether into another test-tube, and filter the solvent into a small watch-glass through a small dry filter. In this way the most of the water will be removed from the ether. Allow the ether to evaporate spontaneously. Dissolve the residue left in a little water, and perform the tests for lactic acid with this solution.

The Digestive Process.—In the process of acid digestion taking place in the stomach the protein substances, such as the albumins and fibrins, are changed to soluble compounds, of which albumoses and peptones are the chief.

As an intermediate product between these compounds, acid albumin is formed. This is produced by the action of the hydrochloric acid on the albumins, and thus the hydrochloric acid serves to transform the food-material into a compound capable of further change by the peptic enzyme.

The primary products of digestion, viz., albumoses and peptones, comprise a series of substances of which little of their real chemical nature is known. In as pure a state as they can be obtained, they are white powdery substances which are readily soluble in water. They give most of the general tests for proteids.

It must be understood that the albumoses and peptones as prepared to-day are not chemical individuals in the strict meaning of the term. They present in themselves certain characteristics of a pure chemical compound, such as constant elementary composition, and react with certain reagents in a well-defined manner; but careful examination shows that differences may be found, showing that they are not strictly homogeneous. The albumoses are roughly classified into

Hetero-albumoses, insoluble in water, but soluble in dilute salt solution.

Protalbumoses, soluble both in water and in salt solution.

Dysalbumoses, formed by the prolonged contact of hetero-albumoses with water.

Deutero-albumoses, soluble in water and in dilute salt solutions. These compounds are not precipitated by saturation with sodium chlorid in neutral solution.

By the digestion of globulins, vitellins, casein, etc., a number of albumose-like substances—the globuloses, vitellooses, caseoses, etc.—are produced.

The albumoses are differentiated and separated from the peptones by their being precipitated on saturation in slightly acid solution with sodium chlorid or with ammonium sulfate.

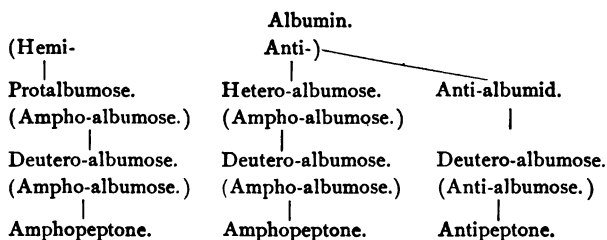
The peptones are substances closely allied to the albumoses, and have very similar properties. They are much more hygroscopic, and are, as a result, easily soluble in water. They are not precipitated by acetic acid and saturated solutions of sodium

chlorid, with picric acid, nor potassium-mercuric iodid, and hydrochloric acid, all of which precipitate the albumoses. They are precipitated with phosphomolybdic acid, absolute alcohol, and with tannic acid.

As a difference also between the albumoses and the peptones may be mentioned that, according to Fränkel, the albumoses, as a rule, contain sulfur, while the peptones do not contain that element.

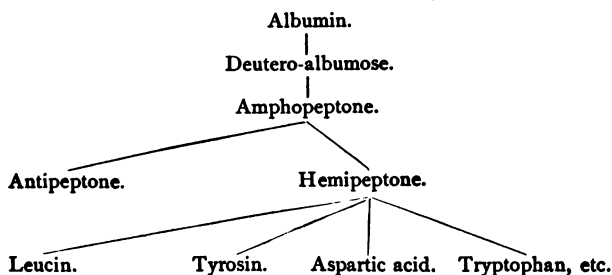
The peptones are in all probability much simpler compounds chemically than the albumoses. For one of them (anti-peptone) the formula $C_{10}H_{15}N_3O_5$ has been given.

The action of the pancreatic juice is much more destructive to the protein molecule than is that of pepsin. While the principal products in ordinary peptic digestion are albumoses (and when the action is pushed to its extreme limit, peptones), the trypsin splits up the molecule into much less complex compounds, such as leucin and tyrosin. The action of peptic digestion on a protein is shown in the following scheme, in which the molecule is divided into two parts, hemi- and anti-, which give different products at the same stage of digestion.



It will be noted that the ultimate products here are amphopeptone and antipeptone. The latter occurs in the smaller quantity.

The action of trypsin is less complicated, although leading to the formation of more compounds. The following scheme shows the action and the compounds resulting :



The final products are the comparatively simple leucin, tyrosin, and aspartic acid, substances belonging to the group of amido-acids, of which glycocoll, $\text{CH}_2\text{NH}_2\text{COOH}$, is the simplest member.

Reactions of the Albumoses.—Use a 2 per cent. solution of Witte's peptone.

1. Heat a few cubic centimeters of the solution to boiling in a test-tube. Coagulation does not take place. Add to the solution a drop of acetic acid, and boil again. The fluid remains clear.

2. Heat a few cubic centimeters with a drop of nitric acid. The precipitate which at first forms dissolves on adding more nitric acid. Heat the solution with a large excess of nitric acid. Note the change to yellow. Put a small amount of the

mixture into a watch-glass, and add cautiously a solution of sodium hydroxid. Note the orange color produced (xanthoproteic reaction).

3. Test the solution with the biuret reaction.

4. Heat 1 c.c. of the solution with Millon's reagent. Note the browning which takes place. To what is this due? Compare the action of potassium hydroxid on glucose.

Pancreatic Digestion.—The functions of the pancreatic juice are threefold.

The most important is the completion of the work commenced by the peptic glands of the stomach in converting the albumins into albumoses and peptones. The second function is diastatic, which is really the following up the action of the saliva on the starch which remained unconverted into dextrins and glucose. The conversion of the fats into an emulsion, and therefore into a form capable of easy assimilation, is the third duty of this secretion, and this is accomplished through the agency of a particular ferment.

As the difficulties surrounding the obtaining of the pancreatic juice in health are so great, nothing is known very definitely regarding its analytic composition. In man, however, it is probable that the secretion is by no means continuous, but depends largely on the stimulation of the nerves controlling the secretion, by the entrance of food into the small intestine. In the case of fistulæ which have been made in animals, and also in man, the absence of food through sufficient fasting is a signal for almost complete arrest of the secretion.

The character of the food is also of influence in the secretion, as it is in the stomach. Acids, mineral and organic, provoke secretion, as do also the fats, while alkalies and alkaline carbonates inhibit. Curiously enough, the quality of the food entering the duodenum, and the relative amounts of the different enzymes secreted, have to a certain extent been made out.

With the ingestion of relatively large quantities of bread the diastatic ferment is increased, while with a milk diet the fat-splitting enzyme is present in the larger amount.

On a strictly proteid diet the proteolytic enzyme is produced in the greater quantity.

An analysis of the pancreatic juice of a dog gave the following composition in percentages:

Solid matter	9.9
Organic matter	9.0
Inorganic matter	0.8
Water	90.1

The fluid is clear and odorless, and is alkaline in reaction.

The action of the proteolytic enzyme is comparable to that of pepsin. While peptic digestion takes place in an acid medium, tryptic digestion is performed only in a fluid with an alkaline reaction. Traces of free mineral acid are sufficient to inhibit completely the action of the pancreatic juice. The amount of free alkali which is most favorable to the action of the enzyme is equal to about 0.3–0.4 per cent. of sodium carbonate.

Pancreatic digestion is a much more energetic process than peptic digestion, and in consequence the original proteids submitted to the action of the enzyme are more completely broken down than in the stomach. Besides the albumoses and peptones which are also formed in the stomach, leucin, tyrosin, lysin, lysatinin, and even ammonia are produced (see page 65). The relative amount of peptone to albumose is also very largely increased, and as the former class of compounds is supposed to be the further stage in the digestive process, this also points to the energy of the tryptic process as against the peptic.

Steapsin, the fat-splitting enzyme of the pancreas, has not only the power of converting fats into the fatty acids and glycerin in a manner analogous to that of saponification, but of emulsifying fats and rendering them capable of absorption by the glandular structures of the bowel wall.

The diastatic ferment, or pancreatic diastase, is closely allied to the ptyalin of the saliva. It acts on both cooked and uncooked starches. Among the products of its action on starch are the dextrans and the sugars maltose and isomaltose. But little glucose is formed.

Examination of the Pancreatic Fluid.—Use the pancreatic fluid made by bruising fresh pancreas with glycerin, and diluting the mixture with water rendered alkaline with sodium carbonate. The solution after filtering must be protected from putrefaction, which it readily undergoes, by the addition of thymol or of chloroform-water. What

answers the purpose equally well, and which is not so subject to putrefactive change, is desiccated pancreas powder, a solution of which, 2.5 grams in 1000 c.c. of chloroform-water to which have been added 3-4 grams of sodium carbonate, gives an active digestive fluid.

Take 25 grams of fibrin which has been washed free from blood-pigment, or which if previously preserved in glycerol should be washed free from it and treated for some time at ordinary temperature with physiological hydrochloric acid (0.3 per cent.).

After washing the last traces of the acid from the fibrin the protein is ready for digestion. The fibrin is digested at 40° C. with the digestive fluid prepared above, 100 c.c. of the fluid being used. The digestion is allowed to proceed for forty-eight hours, the mixture being shaken from time to time. The mixture is then boiled, and acetic acid is added till no further coagulation takes place. The amount of acetic acid necessary will be small. The mixture is filtered through a folded filter. The residue consists of coagulated albumin. The filtrate contains peptones, a small amount of albumoses, tyrosin, leucin, and the other products of digestion, together with tryptophan, which is constantly present in the energetic digestion of proteids. This latter substance is distinguished by giving a beautiful blue color when treated with bromin-water.

The filtrate is evaporated on the water-bath to about 10 c.c., and allowed to stand in a cool place. White bunches of tyrosin crystals will be observed

on examining with the microscope. These are filtered off, and washed with a little water. The second filtrate is evaporated to as small a volume as possible, and set aside preferably in a vacuum desiccator. The residue consists of peptone and a certain amount of leucin. Transfer a small quantity of the residue to a microscopic slide, and examine. Small globules will be found, which on careful examination will be seen to have a radiating structure. These are the globules of leucin (Fig. 10). The residue will also respond to all the tests for peptones.

Tyrosin.—As the amount of tyrosin formed in this experiment is small, care must be taken, in performing the following experiments, to use as minute a quantity as possible consistent with obtaining the reactions.

1. Place a minute quantity of the crystals in a watch-glass over a sheet of white paper. Touch the particle with a drop of concentrated sulfuric acid, and warm for some time over a small flame. Add small fragments of barium carbonate little by little till the acid is neutralized. Add to the mixture a drop of a very dilute solution of ferric chlorid. A violet color will be produced, easily seen on the white barium sulfate in the watch-glass.

2. Heat another fragment with concentrated nitric acid in a watch-glass, and evaporate off the excess of nitric acid till the residue is quite dry. The color of the residue will be light yellow. Moisten this residue with a drop of sodium hy-

droxid solution. The yellow color gives place to a deep orange-red.

The separation of the leucin from the peptones is difficult, and may not be attempted.

Leucin is prepared in the laboratory by heating horn shavings with dilute sulfuric acid (Fig. 10). Use for the following tests leucin prepared in this way.

1. Place a small quantity of leucin in a glass tube open at both ends. Heat carefully. Note the

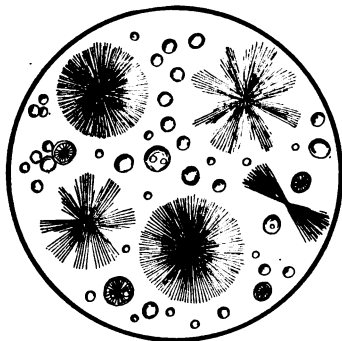


FIG. 10.—Leucin and tyrosin crystals (Salinger and Kalteyer).

sublimate which is formed. Examine with the microscope. Note also the odor of amylamin formed by the distillation of the leucin.

2. Place a few crystals of leucin on platinum foil. Add a drop of nitric acid, and heat carefully till the acid is dissipated. Compare the residue with that given by tyrosin. Add sodium hydroxid solution to the residue. Observe that instead of

the orange residue formed with tyrosin a clear solution is formed, in which oily globules float.

The Tryptophan Reaction.—Add to the solution of the residue of peptones and leucin a few drops of bromin-water. Note the formation of a blue color.

The Diastatic Action of Pancreatic Juice.—Make a small quantity of a solution of soluble starch (page 22).

Digest 1 gram of pancreatic powder with 50 c.c. of water for some time at 40° C. Mix equal quantities of the starch solution with the pancreatic mixture, and digest at 40° C. till the starch solution loses its opalescent appearance and becomes clear. Test the mixture for starch with iodine solution. Is starch present? Is erythrodextrin in the solution? What has happened?

The Action of the Pancreas on Fats.—For this purpose the pancreatic powder used in the foregoing experiments will not answer, and recourse must be had to fresh pancreas. The organ is finely minced, and made up with a little water to a thin mass. One part of this mixture of pancreas and water is heated in a test-tube, the other is allowed to act on the fat before the use of the heat.

A few drops of olive oil are placed in a test-tube with 2 c.c. of water. A drop of dilute sodium hydroxid is added. An equal volume of the pancreatic fluid is now added and the reaction of the mixture tested with litmus-paper. If the reaction be acid, the alkaline reaction is restored with

dilute sodium carbonate solution. Digest the tube at 40° C. for some time. Test the reaction with litmus-paper. It will be found that the reaction has become acid from the decomposition of the glyceryl trioleate into free oleic acid and glycerin. Place along with the tube containing the olive oil and the fresh pancreas solution a similar tube which contains oil with the extract of the pancreas which has been heated to 100° C. Note that the reaction of this mixture does not change. Heating has destroyed the fat-splitting power of the pancreas.

The Bile.—The bile is a clear-yellow to yellowish-green fluid of a specific gravity varying from 1010 to 1040. The amount which is secreted by the human subject in the course of the day is dependent on the amount and kind of food taken. The quantity has been variously estimated from 514 to 950 c.c. in the twenty-four hours. Analyses place the amount of water in the bile at 96–97 per cent. The solids range from 3 to 4 per cent.

The solid matter contained in the bile is made up of mucin, coloring-matter (bilirubin, etc.), bile salts, cholesterin, soaps, lecithin, and inorganic salts. Of these, the biliary coloring-matters and the bile salts are perhaps the most important.

The biliary coloring-matters are the most characteristic substances found in the bile, and form an exceedingly complex group of substances. Some of these are derivatives of others, and are formed by processes of oxidation.

Bilirubin is the chief coloring-matter in the bile,

and is closely allied, on the one hand, with the hemoglobin found in the blood, and, on the other, with urobilin, the normal coloring-matter of the urine. Bilirubin is a red coloring-substance which is probably formed from hematin. On gentle oxidation it yields biliverdin, the next member of the group, and on pushing the oxidation further bilihumin, bilifuscin, and a number of other coloring-matters have been described as being obtained. It is on this property of oxidation that most of the tests for the detection of bile-pigment depend. Bilirubin is difficultly soluble in most solvents, chloroform being an exception. Its coloring-power is so great that a solution of 1 part in 50,000 has a perceptible yellow tint when observed in a column 15 mm. long.

The spectra of bilirubin and the other biliary pigments are not characteristic.

The salts of the biliary acids are those of taurocholic and glycocholic acids. In human bile the former is present in the greater quantity. Glycocholic acid is easily broken up by treatment with acids or alkalis into glycocoll and cholalic acid.

Both glycocholic acid and taurocholic acid form insoluble lead salts on treatment with lead acetate. If the lead salt be decomposed with sodium carbonate, the sodium salts are formed. Of these, the sodium glycocholate is soluble in alcohol. Hence by heating the mixture of sodium taurocholate and glycocholate with alcohol the glycocholate is removed. This property is used in the separation of the two acids. When the bile acids are treated

with sulfuric acid and sugar, or, what is their equivalent, with a solution of furfural, a play of colors takes place, on which the detection of the biliary acids depends. The color produced is at first a bright red, which changes at ordinary temperatures to a bluish violet in the course of a few hours. This color is also distinguished by a characteristic spectrum, in which two absorption-bands are seen, one near E and the other close to F. On account of the number of other substances giving the same color-reaction (albumin, oleic acid, morphin), this reaction is not so useful for the recognition of biliary acids in complex fluids, and therefore for bile itself, as would at first appear. It is also dependent to some extent on the proper relation between the sugar solution and the amount of acid used. The purity of the reagents also affects the reaction. On this account it has been recommended to use a solution of furfural instead of the sulfuric-acid-sugar mixture.

Isolation of the Biliary Acids.—Take 50 c.c. of bile, and add sufficient absolute alcohol to precipitate completely the mucinous compounds. Filter, and evaporate the filtrate in a water-bath nearly to dryness, after adding half the volume of water. Dissolve the residue in 200 c.c. of water, and add lead acetate solution so long as a precipitate forms. The precipitate consists of lead taurocholate and glycocholate. Filter off the precipitate and wash with water. Bring the precipitate into a flask by piercing the point of the filter-paper with a glass rod, and washing the precipitate into the flask with

a stream of water from a wash-bottle. Heat the precipitate with a 10 per cent. solution of sodium carbonate. The lead salts are decomposed into the sodium salts, lead carbonate being formed. Filter off the lead carbonate, and evaporate the solution containing sodium carbonate and the sodium salts of taurocholic and glycocholic acids to dryness on a water-bath. Digest the mixture for some time with 25 c.c. of alcohol in a small flask. Filter. The filtrate contains sodium glycocholate. Reserve till later. Dissolve the residue on the filter in water. Add lead acetate, and precipitate the lead salt of taurocholic acid. Filter this off and suspend in water. Pass hydrogen sulfid gas through the hot solution. Lead sulfid is formed, and the taurocholic acid will be obtained on filtering off the lead sulfid, and evaporating the solution to dryness.

The filtrate containing sodium glycocholate may be treated with lead acetate in the same way, and the lead salt decomposed with hydrogen sulfid. Glycocholic acid is obtained. The mixture of sodium taurocholate and sodium carbonate may also be decomposed by treating the solution with sulfuric acid till the solution reacts faintly acid. Glycocholic acid crystallizes out on cooling the solution.

The acid so obtained may be filtered off, washed with a little water, and dried by pressing between folds of filter-paper and preserved.

Recognition of Biliary Acids.—**Pettenkofer's Reaction.**—Dissolve a small quantity of bile in concentrated sulfuric acid in a porcelain dish. Warm

gently, taking care that the solution is not heated over 60° C. (test with the palm of the hand). Add a drop of a 10 per cent. solution of saccharose. Note the formation of a bright-red color, which changes on standing to a bluish violet. The biliary pigments will be taken up in the section on Biliary Calculi.

Biliary Calculi.—Gall-stones are the concretions found in the gall-bladders of all animals. They are fairly common in man, and are of very frequent occurrence in the ox. In man the chief constituent of gall-stones is cholesterin, while in the ox the concretions formed from biliary pigments combined with calcium are the more common. In most instances the calculi are not homogeneous, but are formed of mixtures of cholesterin with pigment. Carbonate and phosphate of calcium are infrequent constituents of gall-stones.

As with urinary calculi, the concretions formed in the gall-bladder have a concentric structure, made up of successive layers of material, which give to the calculus on section an onyx-like appearance. In addition, calculi containing much cholesterin have a radiating structure, due to the crystallization of that compound.

The semi-crystalline appearance of calculi is not due to crystalline form, but is produced by the attrition of one calculus against another. Where single calculi exist in the bladder the external appearance of the calculus is usually smooth, no facets having been formed.

When taken from the gall-bladder the calculi are

soft and waxy in consistency. On exposure to the air they become hard, as an examination of museum specimens will show. The principal constituent of human biliary calculi is cholesterin. It may be easily extracted from them by means of ether, in which it is readily soluble, while the coloring-matter of the calculi is insoluble in that medium. It is a compound which is not only found in biliary calculi, but is widely distributed in small quantity throughout the animal body. It is found in fairly large amount in the tissues of the nervous system. It is also found in semen, pus, etc. It forms the larger part of the fat obtained from the wool of the sheep (lanolin). As existing in sheeps' wool, it has associated with it a substance closely allied to it, ischolesterin, which is distinguished from it by not giving the characteristic reaction with sulfuric acid. It is a monatomic alcohol of the formula $C_{27}H_{48}OH$. It is insoluble in water, soluble in alcohol and ether, and crystallizes from alcohol in fine plates which are characteristic. It has a fatty feel when rubbed between the fingers.

1. Take a small fragment of a biliary calculus in a test-tube. Add about 0.5 c.c. of alcohol. Heat gently, and decant the alcohol into a watch-glass. Allow the alcohol to evaporate, and examine with the microscope the crystalline residue left.

2. Add to the residue in the watch-glass, placed over a sheet of white paper, a drop of sulfuric acid. Note the change in color to blood red, and the transition of the red color on standing to violet.

3. Dissolve a fragment of cholesterin in 1 c.c. of

chloroform. Add an equal volume of sulfuric acid. Note the change of the chloroform solution to blood red. Observe the green fluorescence imparted to the sulfuric acid.

4. Heat a small fragment of a calculus with sodium carbonate solution in a test-tube. The solution is filtered. It contains the biliary pigments

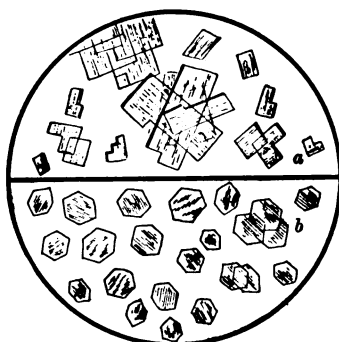


FIG. 11.—*a*, Cholesterin crystals; *b*, cystin crystals (Salinger and Kalteyer).

in the form of sodium salts. Divide the solution into three portions in small test-tubes.

a. To one add a few drops of a solution of calcium chlorid. Calcium carbonate is precipitated, and carries down with it the biliary pigment. Filter off on a small filter, and add to the calcium carbonate a drop of concentrated nitric acid containing nitrous acid. Note the formation of a green color. This changes rapidly to violet, and finally becomes yellow or almost colorless.

b. Allow the contents of the second test-tube to

stand in the air. The bilirubin is oxidized to biliverdin. The solution takes on a green color.

c. Nearly neutralize the contents of the third test-tube with hydrochloric acid. Add to the test-tube an equal volume of concentrated hydrochloric acid containing $\frac{1}{10}$ th of its volume of nitric acid. Note the formation of a greenish-blue color.

MILK.

Chemically, milk consists of a solution of albumins and globulins with milk-sugar, and inorganic salts, and containing fats in an emulsified condition. Fresh milk has a reaction which is known as amphoteric, and has the property of changing either red or blue litmus-paper to the opposite color. It is an excellent culture-medium for micro-organisms, and its composition rapidly changes with the growth of these bodies in it. On heating to boiling, the proteids are not coagulated, but a scum forms on the surface, consisting of a combination of one of the principal constituents of milk, casein with salts of calcium. If this be removed and the milk again boiled, the formation of this substance is again renewed.

The average composition of human and cows' milk is as follows :

	Human milk.	Cows' milk.
Solid matter	11.59	12.83
Casein	1.03	3.02
Albumins and globulins	1.26	0.53
Lactose	3.78	3.69
Ash	0.31	0.71
Water	87.41	87.17
Specific gravity	1.027	1.031

As will be seen from the above analyses, the albumin and globulin are almost twice as great in human milk as in cows' milk, while the casein in human milk is about one-third that in the milk of the cow. The lactose is also greater in human milk, and it is for this reason that lactose is added to cows' milk when that fluid is modified for infant-feeding. For the same reason also the milk is diluted in order to reduce the percentage of casein to that found in the human subject.

As the amounts of the various substances found in milk vary with different individuals of the same species, analyses are made to determine in what proportion a particular milk should be diluted, and lactose and fat added. Even with careful methods, it is a question with practitioners to what extent the so-called modified milk can completely replace that of the human subject.

The most important constituents of the milk are the casein, milk-fat, and lactose. The latter compound has been considered in the section of carbohydrates.

Casein is a proteid containing both phosphorus and sulfur. It is difficultly soluble in water or solutions of salts. It behaves like a fairly strong acid, and therefore forms compounds with metals, such as potassium, calcium, mercury, and silver. The silver and mercury compounds have lately come into prominence as antiseptics. Casein does not exist in true solution in milk, but is probably in a state midway between solution and suspension. Its alkaline salts are easily soluble in water, and

hence opalescent solutions of casein become clear on the addition of an alkali. Casein is also precipitated from its solution by many metallic salts, such as copper sulfate, zinc sulfate, and alum. In the presence of a sufficient quantity of calcium salts which are normally found in milk, it undergoes a peculiar change when treated with the enzymes found in rennet. As occurring in milk, this phenomenon is known as curdling, and is comparable

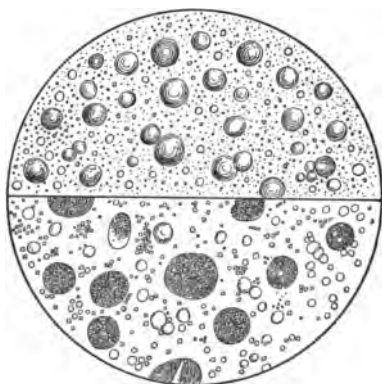


FIG. 12.—Colostrum.

in many respects with the coagulation of blood. The casein on coagulation is transformed into a substance, paracasein, which makes up the principal part of cheese. Lactalbumin is formed at the same time.

The **fats** found in milk exist in the form of a finely divided emulsion of fat-particles, of which there are between 5,000,000 and 6,000,000 in a cubic millimeter. It has been assumed that the

minute particles were surrounded with an exceedingly thin film of albumin which prevented them from segregating. It is probable, however, that the phenomenon is a purely mechanical one, and is due to the surface attraction of the casein solution in which the particles float, preventing them from coming together.

Milk-fat consists chiefly of palmitin, stearin, and olein, the latter being present in the greater quantity. Besides these there are found the glycerids of several of the lower fatty acids, among which are myristic, butyric, caproic, and caprylic acids. To a certain extent to these is due the taste of butter and milk.

General Reactions of Milk.—I. Test the reaction of fresh milk with both red and blue litmus-paper. Note the change in color in both cases.

2. Heat 25 c.c. of milk to boiling in a small beaker. Note the scum formed. Remove the scum, and heat again.

3. Heat to boiling 25 c.c. of milk which has been standing for some days. Note the coagulation of the casein. Test the reaction of this milk with litmus-paper.

4. Add a few drops of acetic acid to fresh milk, and heat to boiling. Note the coagulation of the casein.

5. In a small test-tube shake 10 c.c. of milk with the same volume of ether. Shake vigorously. Does the appearance of the milk change? Decant a portion of the ether into a small watch-glass. Allow the ether to evaporate spontaneously. Note the

residue of fat left. Add 1 c.c. of sodium hydroxid to the mixture of ether and milk in the test-tube. Shake again. Has the appearance of the milk changed? Why?

6. Repeat the guaiac reaction described in the section on Blood (page 46). Note the formation of a blue color as in the case of blood.

7. Take a small quantity of pepsin, and moisten with a few drops of physiological salt solution. Add to this 25 c.c. of milk, and place the test-tube in a beaker of water heated to 40° C. Note the coagulation which takes place in the course of a few minutes. Repeat the test, using equal parts of milk and water. Does the casein coagulate? Why is milk often mixed with water when used as a drink?

8. Heat 5 c.c. of milk with 1 c.c. of potassium hydroxid solution. Note the formation of a brown color. To what is the reaction due?

THE URINE.

Normal urine consists chiefly of a solution of chlorids, sulfates, and phosphates of potassium, sodium, magnesium, and calcium, together with certain organic substances, of which urea is the chief. There are also present in smaller amounts uric acid, creatinin, and coloring-matters.

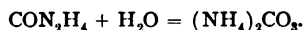
The percentage composition of urine may be said to be as follows :

Total solids	4.8
Urea	2.2
Uric acid	0.03

Hippuric acid	0.026
Creatinin	0.066
Pigment and other organic matter	0.066
Sulfuric acid	0.122
Chlorin	0.500
Phosphoric acid	0.212
Ammonia	traces
Potassium	traces
Sodium	0.33
Calcium	small quantities
Magnesium	" "

The numbers in the foregoing table cannot be taken to represent exactly the composition of normal urine, but they give an approximate idea of the composition of the fluid. It is, of course, understood that the acids and bases do not exist as such in the urine, but are present, as are all salts in aqueous solution, in the form of ions. As will be seen, the greater part of the solids in solution consist of sodium chlorid and urea.

Normal urine is a faintly acid secretion of a not unpleasant aromatic odor and yellow color. Its specific gravity is about 1020. It changes rapidly on standing, and becomes alkaline owing to fermentative change, in which a micro-organism, the *Micrococcus ureæ*, converts the urea into ammonium carbonate.



This change is commonly known as ammoniacal fermentation, and is well advanced in about forty-eight hours, especially if the urine be kept at ordinary room-temperature. This change may take place in the bladder or kidneys themselves, due to

infection through catheterization or other causes. In this case the urine will be strongly ammoniacal when passed. Under these conditions the urine will not be clear, but will have a heavy deposit of ammonium magnesium phosphate. This deposit is very distinct from the precipitate found in strongly acid urines, and which is due to uric acid or to acid urates. It is also to be distinguished from the uric acid deposit by its yellowish-white color and its solubility when the urine is rendered acid.

Urines which contain putrefying organisms have a putrid odor. This is often met with in cases of pyelitis.

The Sample for Examination.—If possible, reliance should not be placed on the examination of one sample of urine, but a series of specimens passed on successive days should be used. It is also strongly advisable that the total amount passed in the twenty-four hours should be collected, and an examination made of the mixed urine. The total amount passed should be noted, for it will be found that the total quantity passed in the twenty-four hours will have a very decided bearing on the diagnosis; and as the urine varies enormously in composition from time to time, the examination of a single specimen passed at one time may lead to erroneous conclusions regarding the urine as a whole. For convenience in measurement the cylinder shown in Fig. 14 may be used. If it be necessary to keep the specimen for some time before it is examined, a small quantity of salicylic acid or

chloroform-water may be added. These will delay fermentative changes. It will be found, however, more advantageous to hasten the examination as much as possible, and to avoid the use of antiseptics.

The Color of the Urine.—Attempts have been made by Vogel and others to construct an arbitrary scale of color for use with the urine. Such a one will be found in Plate 2. This scale is, however,

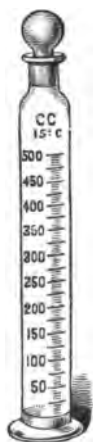


FIG. 13.—Stoppered graduate.

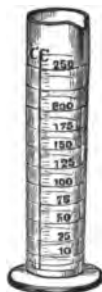


FIG. 14.—Cylindric graduate.

not very satisfactory for the reason that the color of the plates as given in books is viewed by reflected light, while the color of the urine is judged by transmitted light. It is better, therefore, to examine urines by one's self, or better still with another of experience, and form a scale of color which will range from colorless, pale yellow, amber, dark yellow, brownish yellow, brown, and so on, to

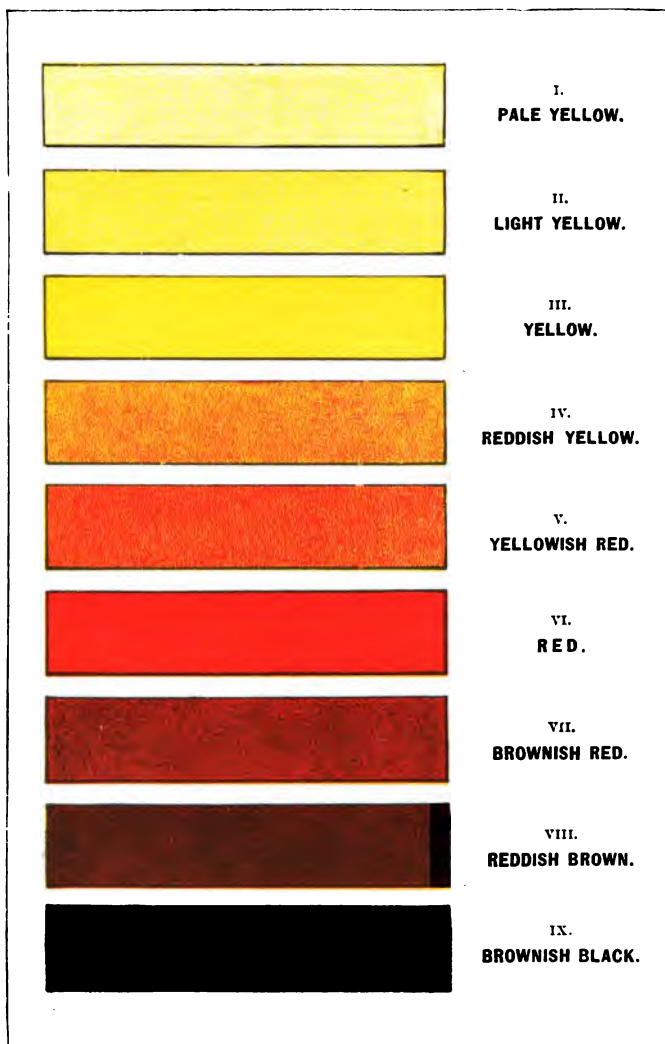


PLATE II.
Scale of Urinary Colors, according to Vogel.

black. It will also be necessary to introduce shades of red, for it will be found that the pigments of the blood are under certain conditions capable of imparting a red color to the urine. The color of the urine is subject to several changes not found in the ordinary scales when certain substances have been taken internally. Salol and many compounds allied to the phenols color the urine a smoky black. Rhubarb, senna, and chrysarobin color the urine a deep red, if the fluid be alkaline, and so simulate the appearance of a hemoglobinuria. This will be detected on the addition of an acid. The color disappears. Santonin produces a bright-yellow urine. Methylene-blue when taken internally produces a urine of a marked bluish-green tint.

The Specific Gravity.—The specific gravity of the urine varies in health with the amount of fluid taken internally and the amount excreted by the skin. Thus in summer, when perspiration is likely to be profuse, the urine may be of high specific gravity; while in winter, when the skin does not act so freely, the specific gravity is likely to be lower.

In the various forms of nephritis, except the acute cases, the specific gravity is diminished; while in glycosuria, even when large amounts of urine are being passed, the specific gravity rises above normal.

The specific gravity of the urine is determined with a special form of areometer called a urinometer. The most useful form is that of Squibb. This has a limited range, being used only for fluids whose specific gravity varies between 1000 and 1060.

This instrument (Fig. 15) differs from the ordinary form of areometer in having a spindle-shaped bulb, and in having the vessel in which it is immersed provided with flutings. In this way the friction between the sides of the vessel and the bulb of the spindle is reduced to a minimum. It is designed to



FIG. 15—Squibb's urinometer.

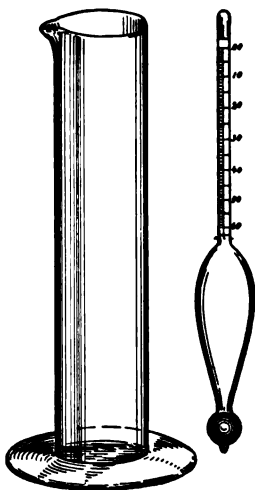


FIG. 16.—Urinometer and urinometer-glass (slightly smaller than one-half actual size) (Ogden).

give its readings at room-temperature (25° C.), and is therefore more easily used than those which require the instrument to be used at 15° C., a temperature which is only to be obtained by cooling the urine. All urinometers as they are obtained from the dealer should be checked by immersion in fluids

of known specific gravity. The 1000 mark may be checked by immersing in distilled water. A fluid of a specific gravity of 1020 may be obtained by dissolving 5 grams of crystallized sodium sulfate in 100 c.c. of water; 10 grams of this salt in 100 c.c. of water will yield a fluid of a specific gravity of 1040. By interpolation a table can thus be constructed, giving the error of the instrument at any part of the scale. This method will yield sufficiently accurate results for ordinary work.

The urine is placed in the vessel and the urinometer floated in it. Care must be taken to have sufficient urine so that the bottom of the spindle is above the bottom of the urinometer vessel. It is better to have sufficient urine so that the surface of the fluid is level with the top of the cylinder. In this way it is easier to make the readings. In reading the urinometer, the upper part of the urine, where it is drawn up on the scale of the urinometer, is taken as the indication of the reading. This is shown in Fig. 17. The urinom-

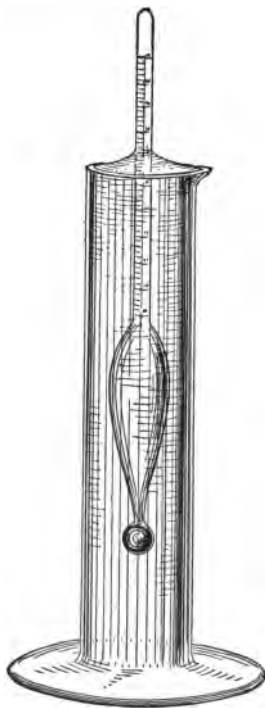


FIG. 17.

eter should be introduced gently into the cylinder, care being taken to exclude bubbles of air, which will increase the specific gravity of the urine. The fluid must also be free from froth. The urinometer is gently pushed down into the fluid and allowed to come to rest, and the upper level of the urine on the scale read off. It is necessary to have the eye on a level with the surface of the liquid, for if the eye be above the level, an error will be made in reading the specific gravity too high.

The Reaction of Normal Urine.—The reaction of normal urine, taken as a whole for the twenty-four hours, is acid. A specimen taken a few hours after a meal is alkaline. The alkalinity of the urine may either be fixed or volatile. The former is probably due to traces of sodium or potassium carbonate; the latter, to ammonia. If one warm the litmus-paper which has been turned blue by the urine over a free flame, or better in a water-oven at 100° C., the red color returns in the case of volatile alkalis, while with fixed alkali the blue color persists.

The administration of many substances has a marked effect on the reaction of the urine. Alkaline salts, the carbonates and borates, the salts of benzoic, citric, salicylic, and tartaric acids, render the urine alkaline. A full meat diet and mineral acids render the urine acid in reaction.

The yellow color of the urine obscures the change in color when litmus is added to the urine; hence the reaction is always ascertained with litmus-paper. To obtain sharper results, it is recom-

mended to use the plaster-of-Paris plates described in testing the reaction of blood.

The Determination of the Total Acidity of Urine and the Preparation of Normal Solutions.—The quantitative estimation of substances found in the urine may be made by isolating the substances and weighing them directly, or by making use of what are known as volumetric methods. The former, which give the only accurate results in many instances, are used as little as possible in clinical work, because the time which the analyses take is considerable, and an amount of skill is required for their performance which is not often obtainable in a course in physiological chemistry. Volumetric methods are hence preferred. Many of these methods do not give, even under the most favorable circumstances, results as close to the absolute as those given by the more complicated way; but for clinical purposes a series of estimations done from day to day is much to be preferred as a means of prognosis or diagnosis to a single determination done more accurately.

Volumetric Methods of Analysis.—The basis of all volumetric work is the law of definite proportions, which states that all substances react with one another in definite masses by weight. If, therefore, one is able to tell the amount of acid used in forming a salt with an unknown quantity of a base, one will be able to tell by a simple calculation the amount of the base present.

In making a standard solution, one dissolves a definite weight of a substance in a known amount

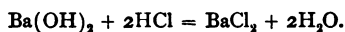
of water. Hence each cubic centimeter of the solution corresponds to a definite weight of the pure substance. Therefore, when one adds a certain amount of the solution, it is equivalent to adding a known weight of the pure reagent.

The solutions used in physiological chemistry are mostly those known as normal solutions. A normal solution is one which contains 1 reacting weight of the reagent in grams in 1000 c.c. of the solution. For example: A normal solution of hydrochloric acid contains $H + Cl = 36.5$ grams of hydrochloric acid in 1000 c.c. Hence, each c.c. contains $\frac{36.5}{1000} = 0.0365$ gram in each c.c.

In this case the molecular weight of hydrochloric acid has been used. In other cases the half or one-third of that weight is employed. For example, normal sulfuric acid does not consist of $H_2 + S + O_4 = 98$ grams of H_2SO_4 dissolved and made up to 1000 c.c., but of half that quantity, viz., 49 grams. This is because sulfuric acid is a dibasic acid, and 98 grams of that substance would neutralize twice the normal molecular weight of a monacid base such as potassium hydroxid:



Similarly, the normal weight of a diacid base, as barium hydroxid, is not 171, as might be imagined, but 85.5, because in a reaction with a monobasic acid, such as hydrochloric acid, the reaction is of the following order:



Therefore 36.5 grams of hydrochloric acid do not require 171 grams of barium hydroxid for neutralization, but 85.5 grams.

As normal solutions are often too strong for convenient work in physiological chemistry, they are made one-tenth of that strength. These solutions are called "decinormal," and are usually expressed by the symbol " $\frac{N}{10}$."

Before using a volumetric method, one should have a very clear idea of the strength of the solutions being employed, and of the exact amount of substance which is added when 1 c.c. of the reagent is added; and, most important of all, of the reaction which is taking place when the reagent is added to the fluid to be tested.

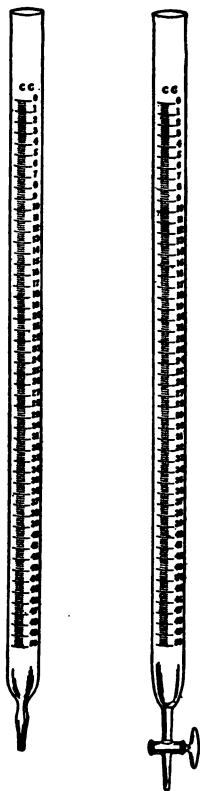
Before the standard solution is added, one may consider the substance which one is estimating as present in excess. Directly the reagent is added a certain amount of the substance is used up in the chemical reaction. If one uses up the whole of this, then on adding more of the reagent there would be no more of the substance present on which it could act, and the reagent, therefore, would be present in excess. Hence it is necessary to know the exact point at which the reagent is added in excess. This is observed by means of indicators. An indicator, as its name implies, is a substance which when added to the solution gives a visible indication of the point at which the reacting substance is present in excess. The indicators employed are of various kinds, suited to the partic-

ular purpose for which they are used. For example: If one adds a few drops of an alcoholic solution of phenolphthalein to a solution of an acid, no color is developed in the solution so long as the faintest trace of acid is present. If, however, an alkali be added so that it neutralizes all the acid and is itself in excess, a fine red color appears in the solution. In the same way, if one adds a solution of silver nitrate to a solution containing chlorids, and to which have been previously added a few drops of a neutral solution of potassium chromate, no changes take place so long as the soluble chlorids are present in excess. If now sufficient silver solution be added to remove all the chlorids in the form of insoluble silver chlorid, and the silver nitrate be in excess, a bright-red color will be observed, owing to the formation of silver chromate, which has that color.

Standard solutions are usually made up by weighing out the required amount of the pure substance, and making up the solution to 1000 c.c. The flask used for that purpose is the one shown in Fig. 22. This has a mark on the neck which indicates the content of the flask. Distilled water is poured in till the lower part of the meniscus touches the mark. These flasks are made in various sizes to contain 110, 250, 500, and 1000 c.c.

When it is required to measure out a small quantity of a solution, use is made of a pipette (Fig. 20). This consists of a small bulb to either end of which are attached narrow tubes. The lower tube is drawn out to a point, and on the upper tube a

mark is etched in the same way as is done with the measuring flask. On introducing the pipette into the liquid, and gently sucking with the mouth, the



FIGS. 18, 19.—Burettes.



FIG. 20.—Pipette.

liquid is drawn up into the pipet. When the liquid rises above the mark the upper end is closed quickly with the finger, and the excess of liquid

above the mark is allowed to flow out till the lower part of the meniscus is exactly on a level with the mark. The pipette is then held over the vessel to which it is intended to transfer the liquid, and the finger removed. The liquid flows out, and after removing the last drop by touching the side of the vessel with the point of the pipette, it is taken away.

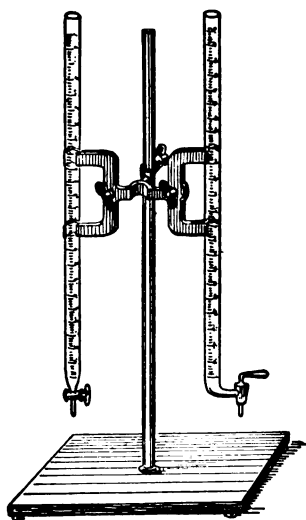


FIG. 21.

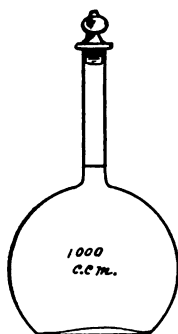


FIG. 22.

Exactly the amount which the pipette is to deliver will have flowed into the vessel. Convenient sizes of pipettes for physiological work are those delivering 5, 10, 25, and 50 c.c.

In titrating solutions, where it is required to add an unknown amount of liquid, and afterward to ascertain the amount added, a burette is employed

(Figs. 18, 19). This consists of a straight tube provided with a stopcock at the lower end, or with a rubber tube which can be opened and closed at will with a spring pinchcock. The former is the more convenient, as being made of glass it is not attacked by the solutions contained in the burette. It must, however, be cleaned immediately after use, as the glass cock has a tendency to stick, whereby the burette will be rendered useless. The straight part of the burette is graduated so that between two marks it will contain 50 c.c. This space is further graduated into 500 divisions, so that each division represents $\frac{1}{10}$ c.c. In using the burette, the apparatus is filled to the uppermost mark, with the lower part of the meniscus touching the zero line. So much of the liquid is run out as is desired; and the amount read off on the graduation on the instrument. The method of reading the level of the liquid in burettes, and, in fact, in all volumetric apparatus, will be seen by examining Fig. 17.

The Estimation of the Total Acidity of Urine.—This operation may be carried out in several ways, but the principle of all these is the same. Two standard solutions are required.

Standard Decinormal Oxalic Acid.—This is made by weighing out 6.3 grams of pure crystallized oxalic acid, and diluting to 1000 c.c. As oxalic acid $(\text{COOH})_2$ is a dibasic acid, but one-half the molecular weight is used.

Standard Decinormal Potassium Hydroxid.—As potassium hydroxid is extremely hygroscopic, it cannot be weighed out directly. Recourse must be

had to other means to make the solution of the exact concentration. A sufficient quantity of pure stick potassium hydroxid is dissolved in 1100 c.c. of water to make a solution stronger than decinormal. This is found to be about 10 grams. Were the potašh free from water, and could be weighed out, but 6.5 grams would be required.

A clean and dry burette is taken, and filled with a standard solution of oxalic acid. 10 c.c. of the potassium hydrocid solution are measured by means of a pipette into a beaker, and diluted with 100 c.c. of water. The solution is colored blue with a few drops of litmus solution, and the oxalic acid delivered into the beaker, with constant stirring by means of a glass rod. When the blue color shows signs of changing, the acid is added drop by drop, till at last the blue color is permanently exchanged for red.

The amount of oxalic acid is noted in the burette. It will be found that this amount is somewhat greater than 10 c.c. It is therefore clear that the potassium hydroxid solution is stronger, volume for volume, than the solution of oxalic acid. The former must hence be diluted till the two are equal. If one subtract 10 from the amount of oxalic acid added, he will have the amount of water to be added to each 10 c.c. of the hydroxid solution to make the two equal. By multiplying this number by 100, the amount necessary for 1000 c.c. will be obtained. 1000 c.c. of the potassium hydroxid are now measured off, and the requisite volume of water added. The solution is mixed thoroughly,

and the new solution of the base titrated. If the operation has been performed carefully, it will be found that exactly 10 c.c. of oxalic acid will be required to neutralize 100 c.c. of the potassium hydroxid.

Example.—Weighed out 10 grams of potassium hydrate, and made up a solution to 1100 c.c. Took 10 c.c. of the solution, and diluted with 100 c.c. of water. Titrated with standard $\frac{N}{10}$ oxalic acid, using a few drops of litmus solution as an indicator. Required 11.8 c.c. of the acid to neutralize. Repeated the experiment. 11.8 c.c. required. Hence each 10 c.c. must be diluted with $11.8 - 10 \text{ c.c.} = 1.8 \text{ c.c.}$ of water. Therefore 1000 c.c. of the solution require $1.8 \times 100 \text{ c.c.} = 180 \text{ c.c.}$ Added this amount of water to 1000 c.c. of the alkali. Took 10 c.c. of the diluted solution, and titrated again with $\frac{N}{10}$ oxalic acid: 10 c.c. required 10 c.c. of the acid. Therefore this solution of potassium hydroxid is decinormal. Each cubic centimeter contains 0.0056 gram of KOH.

Measure out with a pipette 50 c.c. of urine into a beaker, and add the standard solution of potassium hydroxid from a burette. Test the reaction from time to time by taking out a drop of the urine and touching it to a piece of red litmus-paper placed on a clean piece of filter-paper. The spot touched must always be free from the drops of urine which have been previously added. For this reason it is better to distribute small pieces of litmus-paper on

the filter-paper and use a perfectly fresh piece for each test. When the amount of alkali required to turn the litmus-paper a faint purple has been added, this amount is read off on the burette. As each c.c. of the standard alkali is equal to the same volume of standard oxalic acid, and each c.c. of the acid contains 0.0056 gram, then by multiplying the amount of alkali used by 0.0056 the amount of acid expressed as oxalic acid in the 50 c.c. of urine will be given. This need only be multiplied by 2, when the amount of acid as oxalic acid in 100 c.c. will be given.

The Estimation of Chlorids in Urine.—For this purpose the following solutions are required :

1. A decinormal solution of silver nitrate. This is made by dissolving 17.6 grams of silver nitrate in distilled water, and making up the solution to 1000 c.c. Each cubic centimeter of this solution corresponds to 0.00585 gram of sodium chlorid.

2. A solution of potassium permanganate made by dissolving 5 grams of the salt in 100 c.c. of water.

3. A solution of 2 grams of neutral potassium chromate in 100 c.c. of water.

10 c.c. of the urine are diluted with 100 c.c. of water and 10 c.c. of the potassium permanganate solution added. The mixture is then boiled, and a flocculent precipitate of manganous hydroxid is thrown down. This also takes out the coloring-matter, and leaves the urine clear for titration. The manganese hydroxid is filtered off and washed with hot water, the washings being added to the filtrate.

This is allowed to cool, and a few drops of the potassium chromate solution added. The silver nitrate is then added from a burette, with constant stirring, and the point at which the solution changes from a pale yellow to a pink observed. The number of cubic centimeters of silver nitrate is multiplied by 0.00585. This gives the amount of chlorin as sodium chlorid in 10 c.c. of the urine. This number multiplied by 10 gives the percentage of that substance in the urine.

The chlorin in the urine may also be estimated by destroying the organic matter by oxidizing it with sodium nitrate. One gram of sodium carbonate and 2 grams of sodium nitrate are added to 10 c.c. of the urine in a porcelain capsule, and the mixture evaporated first over a water-bath, and then over a free flame. The sodium nitrate oxidizes the organic matter, which chars, and finally, on raising the heat, deflagrates. The mass is heated till quite white. Care must be taken not to apply too great a heat, lest the sodium chlorid be volatilized. The mass is dissolved in hot water and filtered. The amount of water used should be about 100 c.c. This solution is titrated as before, and the calculation made in the same way.

The amount of sodium chlorid in normal urine is estimated to be about 1 per cent. In the twenty-four hours the amount excreted by the kidneys is roughly 10-15 grams. This amount is, however, dependent on the amount of chlorin taken in. As the amount in the general fluids of the body is 0.5 per cent., if an amount of chlorids is assimilated

more than sufficient to make up this quantity, it is excreted by the urine. If, on the other hand, an insufficiency of chlorin is used, the deficiency will be found in the urine. In poisoning with phosphorus or with carbon monoxid the amount of chlorin in the urine is diminished; and this also takes place in febrile affection, such as pneumonia, which are not attended with the formation of diarrheas or exudates.

A rough approximation of the amount of chlorin in the urine may be made by adding a drop of a 12 per cent. solution of silver nitrate to 10 c.c. of the urine. If the chlorids be present in excess, a heavy clotty precipitate will form; if the chlorids be diminished, the precipitate will be less coherent, or in marked cases only an opalescent precipitate will appear.

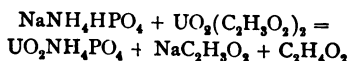
The Phosphates in the Urine.—The salts of phosphoric acid present in the urine are those of the alkalies and the alkaline earths. The amount of phosphoric acid in the urine varies between somewhat wide limits.

The amount excreted per diem by the urine is from 1 to 8 grams. The average is about 3.5 grams. The phosphates are, as the chlorids, a direct result of the amount of phosphoric acid taken in with the food. As the salts of phosphoric acid in the urine are principally the monocalcium and monomagnesium salts, which have an acid reaction, it is to these that the acid reaction of the fluid is due. The quantity of phosphates, except in phosphatic diabetes, has relatively little diagnostic significance.

The Estimation of Phosphates.—The solutions required for the estimation of phosphates:

1. *Standard Solution of Sodium Ammonium Phosphate.*—Dissolve 14.718 grams of this salt in water, and make up to 1000 c.c. Each cubic centimeter of the solution corresponds to 0.05 gram of P_2O_5 . The crystals must be well formed, and dried by pressing them between folds of filter-paper.

2. *Standard Solution of Uranium Acetate.*—As uranium acetate is a deliquescent salt, it is impossible to weigh it out directly. Dissolve 40 grams of crystallized uranium acetate in 1100 c.c. of water to which 50 c.c. of acetic acid have been added. The mixture is filtered and allowed to cool. Fifty c.c. of the standard phosphoric acid are placed in a beaker, and diluted to 150 c.c. with water. The solution is heated to boiling over a wire gauze. The uranium solution is then added from a burette. Combination of the uranium salt with the phosphate takes place according to the following equation,



uranyl ammonium phosphate being formed.

If, before all the phosphate has combined with the uranium acetate, a drop of the mixture be taken out and touched to a drop of a solution of potassium ferrocyanid, no coloration appears. If the faintest trace of the uranium solution be present in excess, a brown color will appear of uranium ferrocyanid. There is thus a method of telling when the uranium solution is present in excess.

Should the uranium solution be exactly equal to the phosphate solution, 50 c.c. would be required to bring about the end reaction. Usually the amount will be less than this, and consequently the amount of solution used from the burette must be diluted to 50 c. c.

Example.—Took 50 c.c of standard phosphate solution, and diluted with 100 c.c. of water. Heated to boiling, and added the uranium solution slowly. 48.5 c.c. were required before the ferrocyanid solution was turned brown. Therefore 48.5 c.c. must be diluted with $50.0 - 48.5 = 1.5$ c.c., and 1000 c.c. must be diluted with $\frac{1000 \times 1.5}{48.5} = 25.6$ c.c. Each cubic centimeter of the uranium solution now corresponds to 0.05 gram of P_2O_5 .

The estimation of phosphates in the urine is carried out in precisely the same manner.

Fifty c.c. of urine are diluted with 100 c.c. of water and heated to boiling, and while in this condition the standard uranium solution is delivered from a burette, the solution being tested from time to time by taking out a drop with a glass rod and touching with a drop of potassium ferrocyanid on a glass plate. When the reaction is given the process is stopped, and the amount of uranium solution read off. In order to obtain the percentage of phosphoric acid in the urine, it is only necessary to divide the number of c.c. of the uranium solution used by 100. Should the urine be strongly alkaline, it is advisable to render it acid with acetic acid before starting the titration.

Total Solids.—The total amount of solids in the urine may be obtained by evaporating 10 c.c. of the urine to a syrup in a weighed porcelain dish, and finally in an oven heated to 60° C. till a constant weight is obtained. On subtracting the weight of the dish from the total weight of the dish and solids, the amount of solids in 10 c.c. of the fluid will be given. This multiplied by 10 will give the total solids per cent.

It is undesirable to dry the residue at 100° C., because a certain amount of decomposition takes place which interferes with obtaining a constant weight.

The amount of solids may also be estimated from the specific gravity by means of Häser's coefficient. If the last two figures of the specific gravity be multiplied by 0.233, the amount of solids per cent. will be given.

Assuming the normal specific gravity of the urine to be 1020, the percentage of solids would be $20 \times 0.233 = 4.6$ per cent. With the total amount of urine excreted per diem as 1500 c.c., the total solids will be $1500 \times 4.66 = 70$ grams. The amount of solids usually assumed to be excreted in the twenty-four hours is 60 grams. This amount would place the normal specific gravity at 1017, supposing the total amount of urine excreted to be 1500 c.c.

Urea.—Urea is the chief nitrogenous constituent of the waste products which is excreted by the kidneys, and it is of correspondingly great importance from a diagnostic point of view. Just what the exact processes are which transform protein

substances into a simple compound like urea are not known, but experimental evidence goes to show that the change takes place in the liver. The quantity of urea passed by the kidneys in the twenty-four hours is roughly 30 grams. An exclusively nitrogenous diet increases it, while any pathologic change in the excretory function of the kidney diminishes it. Hence it is that in cases of

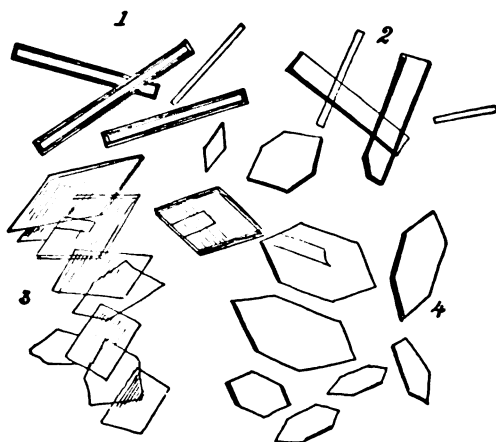
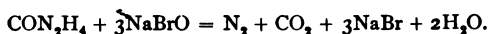


FIG. 23.—1, 2, Urea; 3, smaller scales or rhombic plates of urea nitrate; 4, hexagonal plates.

nephritis the total amount is always less than in health. When metabolism is taking place rapidly, as in fevers, the amount is increased.

Urea itself is a colorless crystalline solid, occurring in stout needles, or in four-sided rhombic prisms (Fig. 23). It is exceedingly soluble in water, and on this account cannot be isolated from the urine alone. It is a very reactive substance and

decomposes readily. This is seen when it is acted upon by the *Micrococcus ureæ*. It takes up a molecule of water and is converted into ammonium carbonate. It is basic in character, and forms sparingly soluble salts with oxalic acid and with nitric acid. It is by means of the nitrate that the compound can be recognized in fluids containing it. On evaporating a fluid containing urea to a small bulk and adding nitric acid, the urea nitrate crystallizes out in well-defined overlapping six-sided plates (Fig. 23). It may also be detected by its effervescence with Labarraque's solution, sodium hypochlorite, or with a solution of sodium hypobromite made by acting on a solution of sodium hydroxid with bromin. In these reactions the urea breaks up, yielding carbon dioxid and nitrogen.



The Quantitative Estimation of Urea.—This is almost universally performed by means of the reaction which has been spoken of above. The method consists essentially of setting free the nitrogen and carbon dioxid by means of hypobromite or hypochlorite, and absorbing the carbon dioxid by means of a strong solution of caustic soda. As the solution of the hypobromite is always strongly alkaline, the evolution of nitrogen and the absorption of carbon dioxid occur simultaneously. A number of forms of apparatus for this purpose have been constructed, of which three will be described:

The first is shown in Fig. 24, and can be constructed out of an ordinary burette. A burette holding

50 c.c. is taken and the lower end connected by means of a piece of stout rubber tubing with a small glass tube which passes through a doubly bored rubber stopper.

The second hole is closed with a piece of glass

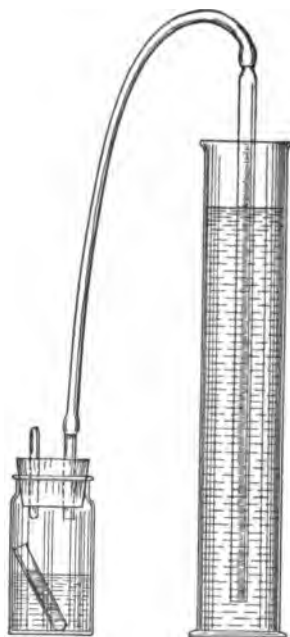


FIG. 24.—Apparatus for the quantitative estimation of urea.

tubing sealed at one end. For this purpose a piece of glass rod answers admirably. The rubber stopper fits into a stout glass bottle holding about 100 c.c. This bottle is provided with a small test-tube which contains about 10 c.c. and is of such a shape

that the tube may be overturned in the bottle by shaking it.

The burette is placed in a long cylinder containing water. To use the apparatus, the flask is filled with 50 c.c. of a solution of sodium hypobromite made by adding 3 c.c. of bromin to 50 c.c. of a 20 per cent. solution of sodium hydroxid. Instead of this solution, the commercial Labarraque's solution may be used. Care must, however, be taken to secure solution of good quality. By means of a pipette 5 c. c. of the urine are measured into the small test-tube, and the tube placed in the flask, care being taken that the tube does not upset and the contents come in contact with the solution in the flask. The cork is now inserted, and the small piece of glass rod closing the other hole in the rubber stopper withdrawn. The water in the cylinder rises into the interior of the burette. The burette is pushed down till the water is on a level with the 50 c.c. mark, and the small glass stopper is inserted into the rubber stopper. The flask is now shaken, and the contents of the tube allowed to mix with the hypobromite in the flask. Gas is given off, and the water in the burette is depressed. When the reaction is over, the burette is raised till the level on the inside and that on the outside of the burette are the same. The volume of gas in the burette is now read off. This is the amount of nitrogen yielded by 5 c. c. of the urine. In accurate estimations the temperature of the water and the barometric pressure are taken into account. The following table will give the weight of a cubic centimeter of nitro-

gen for a limited number of degrees of temperature and for ordinary pressures which will be encountered. To estimate the amount of urea from the nitrogen it is only necessary to multiply by the factor 2.14.

	730.	735.	740.	745.	750.	755.	760.	765.
14° C. .	1.129	1.138	1.145	1.154	1.160	1.170	1.176	1.186
15° " .	1.124	1.133	1.140	1.149	1.155	1.165	1.171	1.181
16° " .	1.119	1.128	1.134	1.144	1.150	1.159	1.165	1.175
17° " .	1.113	1.123	1.129	1.138	1.145	1.154	1.160	1.169
18° " .	1.108	1.117	1.124	1.133	1.139	1.148	1.155	1.164
19° " .	1.103	1.112	1.118	1.128	1.134	1.143	1.149	1.158
20° " .	1.097	1.107	1.113	1.122	1.128	1.138	1.144	1.153
21° " .	1.092	1.101	1.107	1.117	1.123	1.132	1.138	1.147
22° " .	1.087	1.096	1.102	1.111	1.117	1.126	1.132	1.142
23° " .	1.081	1.090	1.096	1.105	1.112	1.121	1.127	1.136
24° " .	1.075	1.085	1.091	1.101	1.106	1.115	1.121	1.130
25° " .	1.070	1.079	1.085	1.094	1.100	1.109	1.115	1.124

To use this table, in which the temperatures are to be found in the horizontal columns and the barometric pressures in the vertical, find the number which corresponds to the temperature and pressure wanted. Multiply this by 2.14 and by the volume recorded, and again by 20. This gives the percentage of urea in the urine.

Example.—5 c.c. of urine used. Volume of gas, 31 c.c. Temperature 18° C., pressure 755 mm. Weight of 1 c.c. of nitrogen at this temperature and pressure = 1.148 mg. $1.148 \times 2.14 \times 31 \times 20 = 1.52$ per cent. of urea.

For clinical purposes the apparatus of Doremus is much used. It is shown in Fig. 25. This apparatus is graduated empirically, and gives results in per cent. direct. It consists of a tube with a pipette

containing 1 c.c. The apparatus is filled to a double mark on the tube, with a 25 per cent. solution of sodium hydroxid. The tube is then inverted, and 1 c.c. of bromin introduced with the pipette. The bromin combines at once with the hydroxid, forming sodium hypobromite. This is facilitated by gently agitating the liquid. The solution is then diluted with water, and the upper part of the tube completely filled with the solution by closing the open end of the tube and inverting it. The pipette is then washed free from the hypobromite, and filled to the mark with the urine to be tested. The beak of the pipette is then introduced into the open end of the tube as shown in the figure, and the urine expressed. The gas disengaged is collected in the closed end, and after the frothing which accompanies the reaction has subsided the percentage of urea is read off. This apparatus does not give very accurate results, as the temperature, pressure, and the varying height of the liquid in the two arms of the tube are not taken into account. But for clinical purposes it is valuable as an indication of the amount of urea in the sample tested.

This apparatus, as modified by Hinds, obviates some of the difficulties of manipulation encountered in the simple Doremus form. The modification consists in having a small arm (Fig. 26) fused to the larger tube, and connected to it by means of a stopcock. The small tube is graduated in tenths of a cubic centimeter. The larger tube is filled with hypobromite as before, and the smaller filled

to the zero mark with urine, the stopcock being closed. On gently opening the stopcock the urine flows into the larger tube and is decomposed, the gas collecting in the upper tube. Any amount of urine that is desired can be allowed to flow in. The upper arm is graduated, so that on using 1 c.c. of

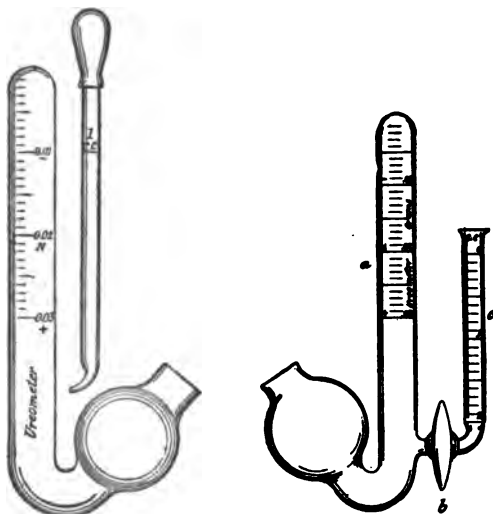


FIG. 25.—Doremus ureometer. FIG. 26.—Hinds' modification of the Doremus ureometer.

urine the percentage is read off directly. If a larger or smaller quantity be used, the reading is corrected for the amount used. The difference in the level of the liquid in the two arms may be got over by placing the tube in a beaker of water so that the level in the inner tube and in the beaker is the same.

Uric Acid.—The amount of uric acid excreted daily by the human subject varies between the wide limits of 0.2 and 1.4 grams. The average amount is about 0.8 gram. According to some observers, the amount relative to the urea is increased in febrile affections. Later work done in this direction would seem to contradict this statement. The amount is increased in the leukemias, where not infrequently 5 grams per diem are excreted, and also in some cases of arthritis. The opinions as to the increase in cases of gout, and rheumatic affections generally, differ. The amounts of the substance excreted seem to vary within the same wide limits as in health.

Uric acid is a very sparingly crystalline solid, which forms, both in the urine and other solutions depositing it, well-defined lozenge-shaped needles, which are often twinned. They may also be recognized in the urine by their dark color, which is due to the tendency which the crystals have to carry down coloring-matter on crystallization.

Uric acid forms a series of salts with the alkalies, and it is in this state that the acid is found for the most part in normal urine. These salts are the urates, which are, in contradistinction to the acid, which is soluble 1 part in 20,000 of water at ordinary temperature, fairly soluble in that liquid. On the addition of an acid to a solution of the salts they are decomposed, and the acid is reprecipitated in its characteristic crystalline form.

Tests for Uric Acid.—1. To a minute fragment of uric acid in a watch-glass add a drop of nitric acid.

Evaporate the acid, and add a drop of ammonia, or allow the watch-glass to come in contact with the fumes of the gas. A splendid purple color results. This test, which is exceedingly delicate, is known as the murexid-test. If a drop of strong sodium

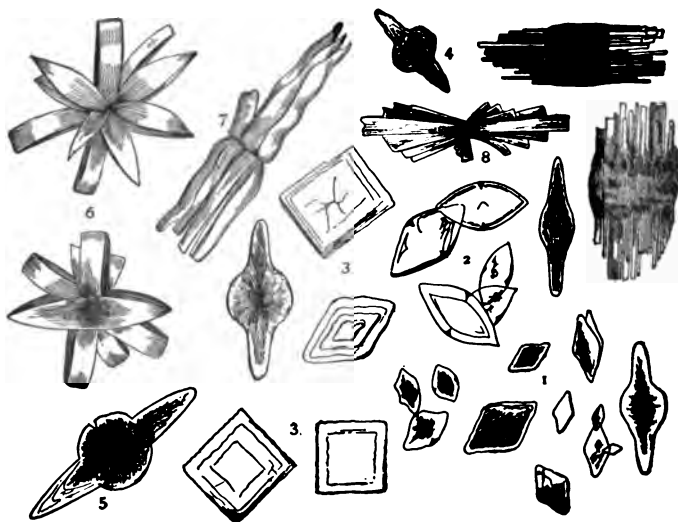


FIG. 27.—Forms of uric acid: 1, rhombic plates; 2, whetstone forms; 3, 3, quadrate forms; 4, 5, prolonged into points; 6, 8, rosets; 7, pointed bundles; 9, barrel forms precipitated by adding hydrochloric acid to urine.

hydroxid solution be added to the purple coloration produced by the ammonia, the violet color changes to blue. On warming the dish the blue color grows fainter, and finally disappears.

2. Dissolve a small fragment of uric acid in a little sodium carbonate solution, and touch a piece

of filter-paper moistened with silver nitrate solution with the sodium urate. An immediate reduction of the silver nitrate takes place, which is evidenced by the blackening of the paper.

The Estimation of Uric Acid in the Urine.—In all cases in which uric acid is to be estimated, the acid must first be isolated. Owing to its insolubility, this is not difficult of performance, but the acid as precipitated is never pure, on account of the coloring-matter and other substances which are included in it when it is thrown out of solution. For this reason it is advisable to precipitate it as ammonium urate, by saturating the solution with ammonium chlorid. The uric acid is separated from this salt, and the acid then either titrated with an alkali or with potassium permanganate. The alkalies are the most convenient standard solutions to use, but they have the disadvantage of a very unsatisfactory end reaction. Tunnicliffe and Rosenheim have obviated this difficulty by employing a standard solution of piperidin as an alkaline solution.

Take 500 c.c. of urine and add 150 grams of solid ammonium chlorid. The ammonium urate will be completely precipitated in two hours at room-temperature, especially if the mixture be agitated during this time. The precipitate is transferred to a filter, and washed with a solution of ammonium chlorid containing 30 grams in 100 c.c. The urate is then washed off the filter with boiling water and decomposed with hydrochloric acid, only a few drops being necessary. The uric acid is precipitated and filtered off. After washing with a

few centimeters of cold water, it is dissolved in boiling water, and the solution titrated with a $\frac{N}{20}$ piperidin solution, using phenolphthalein as an indicator. The $\frac{N}{20}$ piperidin solution is made by dissolving 4.25 grams of the base in water and making up to 1000 c.c. Each cubic centimeter of the solution contains 0.00425 gram of piperidin, and is equal to 0.0084 gram of uric acid. The number of cubic centimeters of the standard piperidin solution used multiplied by 0.0084, and divided by 5, will give the percentage of uric acid in the urine.

Instead of titrating with a piperidin solution, a decinormal solution of potassium permanganate may be used; this contains 3.156 grams of the salt in 1000.

The uric acid solution is washed off the filter with a little potassium hydroxid solution, and diluted with 150 c.c. of water. This is now strongly acidified with sulfuric acid, and heated to boiling.

$\frac{N}{10}$ potassium permanganate is run in from a burette, while the solution is kept at the boiling-point. The end of the reaction is shown by the appearance of a persistent faint pink color.

If 500 c.c. of the urine be used, and the amount of permanganate required to complete the oxidation be 45 c.c., then as each cubic centimeter of the permanganate is equal to 0.0075 gram of uric acid, the percentage of the acid in the urine will be

$$\frac{45 \times 0.0075 \times 100}{500} = 0.056 \text{ per cent.}$$

The normal quantity, assuming that 1500 c.c. of urine are passed per diem, is 0.033–0.05 per cent.

Albumin.—According to many investigators, albumin is a normal constituent of the urine. This is not at all impossible, as the secretion always contains a small number of cells which contain a certain amount of soluble protein substances, which would be recognized were the tests used of sufficient delicacy. Fortunately for clinical purposes, the tests, although of great sensibility, do not, unless under certain conditions, indicate the presence of albumin in normal urine. Hence for clinical purposes albumin may be said to be absent from the urine. This statement does not include the cyclic or physiologic albuminurias which are the subject of so much difference of opinion. In these cases the albumin occurs when the most careful examination fails to reveal any other symptoms of disturbance. Albumin is also found at times of great physical or mental strain, and is usually in these cases associated with the rise in arterial tension.

Albumin is found in solution in the blood, and except in cases of pyuria is derived from it. The two forms of proteins which are present, serum-albumin and paraglobulin, are not distinguished clinically.

The **tests** which serve to detect albumin in the urine depend on the conversion into a modified proteid, coagulated albumin, or into an insoluble compound of the albumin with some metallic salt.

The Boiling-test.—This test is performed with the

filtered urine, which must be perfectly clear. The urine is most conveniently contained in a long and narrow test-tube, and only the upper part (Fig. 28) is heated. In this way the slightest clouding of the urine is made apparent on holding the tube against a black background. The urine must be acid, and nitric acid is most convenient for this purpose. Only a sufficient quantity to render the urine acid should be used. In the presence of larger quantities of the acid a small trace of albumin will be dissolved, and so remain undetected.

Heller's Test.—About 10 c.c. of nitric acid are placed in a small test-tube, and by means of a pipette an equal amount of the urine is floated on the top, so that the two liquids do not mix. The pipette should have the end drawn out to a fine point, and the end should be held as near the surface of the liquid as possible. In this way a sharp line of demarcation is formed between the acid and the urine, and it is on this that much of the success in making the test depends. Should albumin be present, an ivory-looking ring is formed at the junction of the urine and the acid, while smaller amounts will be indicated only on allowing the tube to stand for some time, and examining for an opalescence at the line of contact by holding the tube against a black background.

The fallacies of this test are due to the presence of mucin, and of large quantities of urea and uric acid. The latter two will not give a ring if the urine be previously diluted; while the former is indicated by a cloud which forms, not at the point

of contact, but at a variable distance in the urine layer (Fig. 28).

Many other reagents have been proposed for the detection of albumin in the urine, some of which are of extraordinary delicacy. These all have the disadvantage, from a clinical point of view, in giving precipitates with substances which are found in normal urine. Much more care is necessary in the performance of these tests than in the foregoing. Moreover, should the presence of albumin not be discovered in the urine with the nitric acid test, it may be assumed, unless the clinical features point to the reverse, that the albuminuria is not of very serious importance.

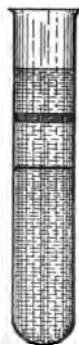


FIG. 28.

Tanret's Test.—Perhaps the most delicate test for the presence of albumin in the urine is the one with potassium mercuric iodid. The test-solution is made by adding a saturated solution of mercuric chlorid to a 10 per cent. solution of potassium iodid. This solution precipitates albumin, and may be used by the contact method, by floating 10 c.c. of the urine on an equal volume of the reagent, or by simply adding 1 c.c. of the reagent to 10 c.c. of the perfectly clear urine. The urine must previously be made acid with acetic acid.

This solution precipitates, besides albumin, alkaloïds, mucin, and other nitrogenous substances

found in the urine. Hence great care must be taken not to confound these with albumin. On the other hand, should no precipitate be yielded by the reagent, one is perfectly justified in concluding that albumin is absent from the urine.

The presence of albumin in the urine may be due to several causes. It may be a sign of inflammatory change, chronic or acute, in the kidneys, and in this case will almost invariably be accompanied by tube-casts. These may be difficult to discover, and, indeed, may be found only after the most careful examination, but they will certainly be present. The introduction of blood into the urinary tract will also be partly shown by the appearance of albumin in the urine, and this may often be the deciding point between a hemoglobinuria and a hematuria. In the cases in which the amount of blood is minimal it may often be impossible to detect the corpuscles, even after centrifuging the urine; but the tests for albumin are so delicate that the albumin from the plasma will be readily detected. As albumin is one of the normal constituents of pus, the presence of this in the bladder in very small quantities will give a positive reaction to the tests for albumin. The albumin in cases of pyuria comes out, on adding alkali, in the form of long glutinous masses. If albumin be introduced into the urinary tract as a result of a cystitis, ureteritis, or urethritis, it is impossible to tell by purely chemical means if the kidneys themselves are not furnishing an additional amount.

There are two methods of **estimating the amount** of albumin in urine:

Estimation by Weight.—One hundred c.c. of the urine are introduced into a beaker, and acidified with a drop of acetic acid. The beaker is placed in a vessel of water kept at 100°C . The beaker is allowed to remain at this temperature for thirty minutes. If the albumin is not coagulated into large flakes, the addition of a couple of drops of acetic acid is allowed. The albumin is filtered through a 11 cm. filter which has been previously dried to constant weight in an air oven at 110°C . The albumin is washed with water, alcohol, and a little ether, and the filter and its contents dried at 110°C . to constant weight. The difference between the first weighing and that with the albumin gives the weight of the albumin, and also the percentage. In very accurate estimations the filter is incinerated and the weight of the ash subtracted from the weight of the albumin.

For clinical purposes the estimation of the albumin is carried out by *Esbach's method*.

The urine is filtered, and a sufficiency to fill the tube (Fig. 29) to the mark U is introduced. On this is poured the reagent, which is made from the following formula:

Picric acid	20 grams.
Citric acid	10 "
Water	1000 c.c.

The reagent and the urine together fill the tube to the mark R. The tube is closed with a cork, and

the two fluids mixed by inclining the tube several times. Care must be taken not to agitate the tube violently, else some of the flakes of albumin will be included in the froth. The tube is now allowed to stand for twenty-four hours, and the height of the sediment on the graduations of the tube read off. These correspond to parts of dried albumin in 1000 parts of urine. If the urine be found to contain more than 0.7 per cent., it should be diluted with an equal volume of water, and the result obtained multiplied by 2.

The method is not satisfactory from a scientific point of view, for the specific gravity of the urine and the temperature exercise a considerable influence on the volume of the precipitate. As a comparative method, which can be easily carried out in the physician's office, it answers very well.

Peptonuria.—The qualitative examination of the urine for peptones and albumoses is often of importance, for, like indican, the substances are found in the urine in cases in which large quantities of pus are being formed in the system and simultaneous disintegration is taking place. Peptones are never present in



FIG. 29.—Esbach's albuminometer.

large amounts in the urine, and therefore a fairly large volume of the urine must be taken for examination.

Hofmeister's modification of Salkowski's method may be used. Fifty c.c. of the urine are precipitated with a solution of phosphotungstic acid, after being acidulated with 5 c.c. of concentrated hydrochloric acid. The precipitation is brought about by boiling the solution and adding the phosphotungstic acid in sufficient quantity to produce a flaky brown precipitate. On continued boiling the precipitate becomes adherent to the sides of the beaker, and the solution may be poured away. The brown precipitate is washed with a little water, and dissolved in a dilute solution of caustic potash. The potassium hydroxid solution is heated till the color, which at first is blue, changes to a light yellow. The solution which contains the peptones is transferred to a small test-tube, and a drop of a dilute solution of cupric sulfate added. A beautiful reddish-violet color is produced if peptone be present.

The solution of phosphotungstic acid is made by dissolving 20 grams of pure sodium tungstate in 100 c.c. of water. Enough syrupy phosphoric acid is added to give the solution a strongly acid reaction. The mixture is filtered and allowed to stand, and the solution poured off from any insoluble residue.

Albumoses are also precipitated with phosphotungstic acid, and also give the biuret-test. As they are identical for clinical purposes, no attempt is made to distinguish them. The albumoses may, how-

ever, be tested for by removing all the coagulable proteids with acetic acid and heat, as was detailed in the quantitative estimation of albumin, and treating the filtrate with potassium ferrocyanid solution. This gives a white cloud if traces of albumoses be present.

Hematuria and Hemoglobinuria.—The presence of one or more of the constituents of blood in the urine may be taken to indicate a pathological condition in some part of the urinary tract; it is, therefore, important to be able to recognize it with as great surety as possible. Urine containing blood may be recognized by its color, which will vary from a slightly smoky yellow to deep red or black. Two pathological states may be distinguished:

1. *Hemoglobinuria.*—In this state only the coloring-matter of the blood is present, while the organized elements are absent.

2. *Hematuria.*—All the elements which go to make up the blood—corpuscles, red and white, and blood-coloring matter—are present. Hematuria thus implies hemoglobinuria, while the reverse is not the case.

The tests for **hemoglobin** are in most respects the same as those given in the section on the Blood. The changes which have been made are to adapt them for the urine.

1. To a sample of urine add a dilute solution of potassium hydroxid, and boil. A precipitate of earthy phosphates is thrown down, which differs from that in a specimen of normal urine in being colored brown by the blood-pigment. The phos-

phates are filtered off, and transferred to a microscopic slide. A drop of glacial acetic acid is added, and a tiny crystal of sodium chlorid. Heat gently after covering with a cover-slip. The characteristic crystals of hemin will appear.

2. The guaiacum-test is performed, using 10 c.c. of urine. The details of the test are the same as those given on page 46. This test is particularly valuable in urinalysis, as the blood will often be indicated in the nephritis following scarlet fever

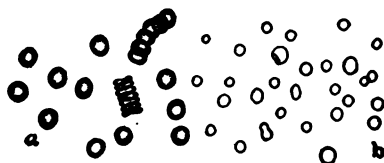


FIG. 30.—Blood-corpuscles: *a*, normal; *b*, abnormal (Ogden).

before any other symptoms of the process will be apparent.

Hemoglobin will also be detected in the urine by means of the spectroscope. The form which appears in the urine is either oxyhemoglobin or hemoglobin. The spectroscopic appearance of these two forms has been described on page 46. In cases of poisoning from potassium chlorate the spectrum of methemoglobin has been recognized in the urine.

Hematuria.—In this condition the corpuscles will be recognized. The form which these elements take on is largely dependent on the state of the urine in which they are present. Should the urine be high in specific gravity, the corpuscles will be

found to be crenated, and will have the normal biconcave appearance exaggerated (Fig. 31) on account of the diffusion of the contents of the corpuscle into the urine. With a dilute urine the corpuscles swell up and become almost spheroidal in shape. They also become paler and almost colorless from the passing out of the hemoglobin into the urine.

Glycosuria.—In testing for sugar in the urine,

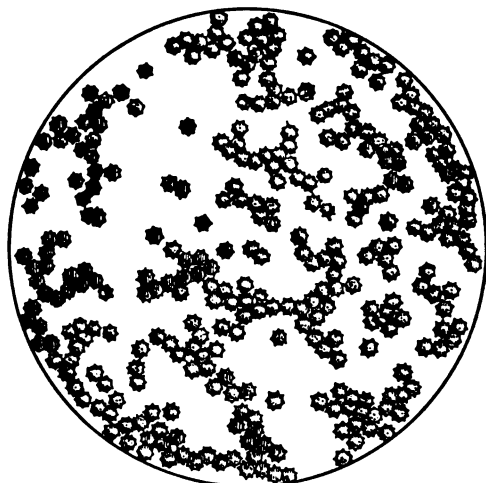


FIG. 31.—Crenated red blood-corpuscles in the urine. $\times 350$.

the tests given in the section on monosaccharids are used. Some of the tests, notably the α -naphtol test, are so delicate that they detect other reducing agents in the urine, and so complicate the reaction. As with albumin, so with sugar, it is a question if in normal urine there is not a small amount of glucose

present. The clinical tests are so arranged, however, that this trace if present is not indicated. In an examination of the urine the conditions which point to the presence of glucose are a high specific gravity and an increase in the total amount passed in the twenty-four hours.

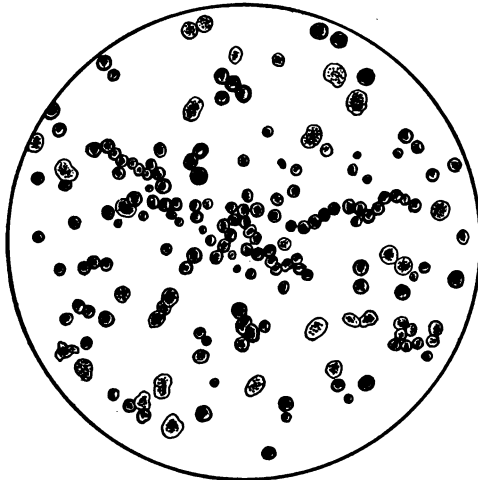


FIG. 32.—Colored and (a) colorless blood-corpuscles of various forms.

Of the tests which have been proposed for the detection of glucose, that of Fehling is the most important. The mechanism of this test has been spoken of on page 14.

The solution is made from the following formula:

Solution 1:

Copper sulfate	34.65 grams;
Water to	1000 c.c.

Solution 2:

Sodium potassium tartrate	173 grams;
Water	350 c.c.

To solution 2 add 600 c.c. of a 10 per cent. solution of potassium hydroxid, and dilute to 1000 c.c.

These solutions should be kept in separate bottles, and mixed in equal parts and diluted with 3 parts of water before use.

Fifteen to 25 c.c. of the mixture should be taken and boiled, and the urine added drop by drop. Small amounts of glucose are recognized by the extremely fine precipitate of cuprous hydroxid, which is yellowish. Larger quantities are indicated by the brick-red cuprous oxid which is thrown down.

The other tests, which depend on the reduction of a metallic solution (Böttger's test, etc.), are of less value. Böttger's test is often complicated by the presence of substances in the urine containing sulfur, which give a black color with the reagent.

The points to be observed in the performance of *Fehling's test* are to add the urine to the diluted Fehling's solution, which must be at the boiling-point, and to see that the mixture is boiled after each addition of urine. It is also of importance to see that not more than 5 c.c. of urine be added, as there are always present in normal urine substances that will respond to the test provided sufficient urine be used.

The most certain means of detecting glucose in the urine is the *phenylhydrazin test* (page 17). The test is performed with urine in identically the same manner as there described. There is but one substance in urine which gives a similar crystalline precipitate with the reagent. This is glycuronic acid. Should any doubt be attached to the test, a

determination of the melting-point of the precipitate will at once decide. Phenylglucosazone melts at $205^{\circ}\text{C}.$, while the melting-point of the compound with phenylhydrazin and glycuronic acid is $104^{\circ}\text{C}.$ Moreover, when the test is performed, as there described, by heating in a water-bath at $100^{\circ}\text{C}.$ for thirty minutes to an hour, the latter compound is decomposed. For this reason the method of heating for a long time is preferable, if slower, than simply to heat over a free flame for few minutes. The limit of the sensitiveness of the test is about 0.3 per cent.

The **quantitative estimation** of glucose in the urine may be made in three ways: 1. By the fermentation method; 2. By titration with an alkaline copper solution; 3. By the polarimeter.

The Fermentation Method.—This is due to Sir William Roberts, and for clinical purposes will be found excellent, as it requires only a good urinometer for its performance. The urine should be made faintly acid with a few drops of a strong solution of tartaric acid.

Two bottles are taken, and each filled with about 200 c.c. of the urine. In one bottle is placed a small quantity of yeast, which should be washed on a filter to free it from fermentable material. The necks of both flasks should be closed with plugs of cotton-wool, and allowed to stand at room-temperature for twenty-four hours. At the end of this time the specific gravity of both is taken. It will be found that the urine to which the yeast has been added has lost in specific gravity, owing to decom-

position of the glucose into alcohol and carbon dioxid:



Each difference of 1 degree between the fermented and the unfermented urine will correspond to 0.23 per cent. of glucose.

Titration with an Alkaline Solution of Copper.—The titration may be carried out with Fehling's solution. Except in experienced hands, this solution does not give very satisfactory results. This is due to the fact that the end-point, where the copper is completely precipitated by the glucose solution, is difficult to recognize. It is better, therefore, to use a solution in which the transition of the blue to a colorless solution is not masked by a heavy precipitate. The most satisfactory solution for clinical purposes is Pavy's solution as modified by Purdy. The composition of this solution is as follows:

Cupric sulfate	4.742	grams;
Ammonium hydroxid (sp. gr. 0.88)	450.9	c.c.;
Potassium hydroxid	23.50	grams;
Glycerol. puriss.	38.0	c.c.;
Water to	1000.0	c.c.

The solution is formed by taking well-formed and clear crystals of copper sulfate, which are powdered and pressed between folds of filter-paper, to free them from adherent moisture. The required amount is weighed out, and dissolved in 200 c.c. of distilled water. The glycerol is added to this solution. The potassium hydroxid is dissolved in another 200 c.c. of water. The two solutions are

mixed, when a copious blue precipitate is formed. The ammonia is then added, and the precipitate is dissolved. Allow the solution to cool to room-temperature, and add water till the mixture measures exactly 1000 c.c.; 35 c.c. of this solution are completely decolorized by 0.2 gram of glucose.

To carry out the test, measure out 35 c.c. of this solution into a 200 c.c. boiling flask, and dilute with 70 c.c. of water. Bring the solution to the boiling-point by heating the flask over a free flame. Fill the burette with the urine to be tested, and allow the urine to flow into the flask, which is kept constantly boiling. This is continued till the solution is seen to lose its blue color. The urine is added more slowly—at last drop by drop. The final change from a very pale blue to colorless is well marked if the flask be held over a sheet of white paper.

The calculation is made in the following way: Knowing that 35 c.c. of the solution are decolorized by 0.02 gram of glucose, the number of cubic centimeters of urine used must contain that amount. Hence, 0.02 divided by the number of cubic centimeters of urine used will give the amount of glucose in 1 c.c. This multiplied by 100 will give the percentage of glucose in the urine.

Example.—8.5 c.c. of urine were required to decolorize completely 35 c.c. of the solution. Amount of glucose in 8.5 c.c. of urine = $0.02 : 8.5 = 0.0023$ gram. Percentage of glucose in the urine = $0.0023 \times 100 = 0.23$ per cent.

Should it be found on the first titration that the urine contains more than 0.5 per cent., it will be

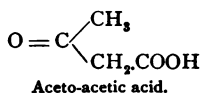
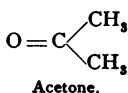
advisable to dilute the urine with 2 to 5 parts of water, and to multiply the result obtained by the corresponding number.

The Polarimetric Estimation of Glucose.—This has been taken up in the section on the Carbohydrates. It is of very great value in hospital laboratories, where the estimation of glucose has to be made a number of times in the day, with as great expedition as possible. In using the saccharimeter with urines containing albumin as well as sugar, it will be necessary to free the urine from the former by adding a drop of acetic acid and boiling, and filtering off the coagulated albumin.

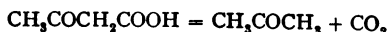
If, as often happens, the urine be clouded from the presence of bacteria, or be too high-colored to permit of a satisfactory reading being obtained, it will be necessary to boil the urine with a mixture of animal charcoal and "kieselgühr" (infusorial earth), and to filter through a dry filter. After this treatment, usually sufficient of the coloring-matter is removed to allow a reading to be taken. If this does not prove effectual, add 25 c.c. of a 10 per cent. solution of lead acetate to 75 c.c. of the urine, and filter off the precipitate, which consists of the phosphates and chlorids of lead, together with the coloring-matter. A dry filter is also used here for filtering. The filtrate is examined with the polarimeter in the usual way. The reading must be corrected for the dilution with the lead acetate solution by multiplying by 1.33 (4-3).

Acetonuria.—Almost invariably associated with glucose in the urine is aceton. It is this substance,

or substances closely allied to it, which give the peculiar fruity odor to the urine in cases of glycosuria. Aceto-acetic acid is also often present, and as these two substances are of importance from the point of view of prognosis, it is necessary to test for them when examining urine which has been found to contain sugar. These two substances are closely related, for aceto-acetic acid may be said to be derived from acetone by the introduction of a carboxyl group, —COOH , into the acetone molecule.



Further, aceto-acetic acid is unstable, and on standing in solution or on boiling loses a molecule of carbon dioxide,



and is converted into acetone. It is therefore necessary in testing for aceto-acetic acid to use perfectly fresh samples of the urine.

Detection of Acetone.—The urine may be used alone; but it is better in most cases to distill 100 c.c. of the urine in the apparatus shown on page 16, after the addition of 10 c.c. of strong sulfuric acid, to prevent frothing. When 10 c.c. of the distillate are collected, they are divided into two parts and submitted to the following tests:

Lieber's Test.—To 5 c.c. of the distillate add 1 c.c. of a strong solution of iodine in potassium iodide (Churchill's or Lugol's solution), and then an

aqueous solution of potassium hydroxid. The latter is added slowly till the color of the iodine is discharged. If acetone be present, a whitish-yellow cloud forms, and the mixture has the characteristic odor of iodoform. When the precipitate subsides and the sediment is examined with the microscope, it will be seen to consist of small yellow needles arranged in the form of rosetts.

Sodium Nitroprussid Test.—To 5 c.c. of the distillate add a few drops of a freshly prepared solution of sodium nitroprussid, and a couple of drops of sodium hydroxid solution. The solution may be colored red from the presence of creatinin, which gives a red color with this reagent. When this color disappears, or commences to be faint, the solution is rendered acid with acetic acid. In the presence of acetone the solution will take on a carmine-red color, which becomes blue on standing.

Aceto-acetic Acid.—As aceto-acetic acid is decomposed, on boiling, into acetone and carbon dioxid, it will be necessary to test for this substance direct in the urine.

To the urine add a few drops of a neutral solution of ferric chlorid. The appearance of a red color indicates the presence of this acid. If the urine be then boiled and treated with ferric chlorid, and no red color be obtained, it will be very good evidence that the red color was due to aceto-acetic acid.

This may be confirmed by making 20 c.c. of the urine acid with a few drops of very dilute sulfuric acid, and shaking with ether. The ether extracts the aceto-acetic acid from the urine. On adding

ferric chlorid to the ether a blood-red color will be given if the acid be present.

Although both acetone and acetic acid give Lieben's test, aceto-acetic acid is the only compound which reacts with ferric chlorid.

Indican.—This substance, which is usually assumed to be the indoxyl sulfate of potassium, is closely allied to indigo, and is found in the urine in cases of wasting disease, where perhaps a large focus of suppuration is present. It is observed also in cases of hyperpyrexia, as in typhoid fever, and in cholera. It is characterized by the ease with which it is oxidized to indigo, and all the tests which have been proposed for its detection rely on this property.

Active chlorin is the most common reagent employed for oxidation, and Jaffé's test is the most satisfactory. The reagent is made by mixing equal volumes of the urine and hydrochloric acid, and dropping into the mixture a little saturated solution of calcium hypochlorite (bleaching-powder). If indican be present, the solution is colored deep blue, owing to the formation of indigo. This deep-blue color will be extracted on shaking the mixture with a few cubic centimeters of chloroform. The solvent becomes slaty blue in color.

Biliary Urine.—The constituents of the bile appear in the urine in two forms—biliary acid and biliary pigment.

Icteric urine varies in color. It is often yellow, the intensity of the color distinguishing it from that imparted by urobilin, the natural coloring-

matter of the urine. Moreover, it not unseldom takes on a greenish tinge, which may be so pronounced as to render the urine opaque, except in thin layers. The biliary pigment has the property of being absorbed by cellulose, a fact which is made use of in one of the tests.

Biliary Acids.—These are detected by Oliver's test, which is a reproduction of a process occurring in the duodenum, when the albumoses are precipitated by the biliary acids in the bile.

Oliver's test-solution is made by dissolving 2 grams of commercial (Witte's) peptone and 0.25 gram of salicylic acid in 250 c.c. of water, and adding 0.5 c.c. of acetic acid. The solution is filtered through a double filter till perfectly bright, and allowed to stand. Three c.c. of the solution are then mixed with 1 c.c. of urine. Should the biliary acids be present in excess, a whitish cloud will appear. Normal urine gives a faint opalescence. It is well in trying the test to compare the opalescence given with the urine, with that obtained with a specimen of normal urine, and, further, to dilute the urine to a specific gravity of 1008.

Bile-pigments.—The pigments are of more importance clinically than are the bile salts, for although the biliary salts are found in small quantity in the normal urine, it may be said that the occurrence of the bile-pigments in the urine is always of pathological significance. The tests for bile in the urine are numberless, and few are satisfactory. Most of them depend on the change in color which the pigment undergoes when an oxidizing agent is

applied to the urine, as has been explained on page 75. Most of these are entirely unsatisfactory when used directly with the urine or with the residue left on evaporation. It is better, therefore, to extract the pigment from the urine, which fortunately can be done readily.

Hüppert's Reaction.—Ten c.c. of the urine are treated with a few drops of a 10 per cent. solution of calcium chlorid, and the calcium precipitated as carbonate by the addition of a solution of sodium or ammonium carbonate. The calcium carbonate carries down with it the bile-pigment. The calcium carbonate is filtered off, and washed with a few cubic centimeters of water, and the residue on the filter transferred to a small test-tube and covered with a few drops of alcohol to which has been added a drop of sulfuric acid. In the presence of bile-pigment the alcohol is tinged a bright green.

Gmelin-Rosenbach Reaction.—The urine is simply filtered through a small filter-paper. Owing to the property which cellulose has of absorbing the pigment, the filter-paper will be tinged with green. A drop of strong nitric acid, containing nitrous acid (made by dropping a tiny crystal of sugar into 1 c.c. of nitric acid), is allowed to fall on the filter-paper. A pale-yellow spot forms, surrounded by a yellow ring, which becomes successively violet, blue, and green from the progressive oxidation of the pigment.

It is a curious feature of icteric urine, that the presence of biliary pigment is invariably associated with the appearance of casts, and a microscopic

examination of the urine will sometimes aid when the tests for pigment are not altogether satisfactory. The casts will usually be found to be stained a light green or yellow by the biliary coloring-matter.

Ehrlich's Reaction.—This reaction has been the subject of much discussion, both as regards its origin and its clinical utility, and as yet investigators are not agreed either as to the substances which give rise to it or as to the pathological circumstances which produce it in the urine. Later observations have not borne out the statement that it is produced solely in the urine of patients suffering from typhoid fever, in so far as several other states accompanied by hyperpyrexia, viz., miliary tuberculosis and infective fevers, will yield a urine that gives the same reaction. It, however, has this value, that in many cases of typhoid the urine will respond to the test before the other symptoms are sufficiently well marked to permit of a diagnosis.

Two solutions are required, which are mixed shortly before using.

Solution 1 :

Hydrochloric acid	15.0 c.c.
Sulfanilic acid	0.5 gram.
Water	500.0 c.c.

Solution 2 :

Sodium nitrite	0.5 gram.
Water	500.0 c.c.

To perform the test, mix 20 c.c. of No. 1 with 0.5 c.c. of No. 2 in a test-tube, and add to this an equal bulk of urine. On the top of the mixture are floated 3 c.c. of strong ammonia. At the junc-

tion of the ammonia and the mixture a splendid scarlet ring will be formed if the test be responded to. On the tube being shaken, the froth which forms will be a carmine red.

Melanuria.—In the urine of patients suffering from melanotic growths is not infrequently found the pigment which constitutes the coloring-matter of these tumors. It is also excreted in the form of a chromogen which is colorless, and which later, on exposure to the air, oxidizes to melanin, which is the coloring-matter itself. The coloring-matter is insoluble in water, but is readily soluble in alkalis. It contains sulfur, iron, and nitrogen. In many instances the urine containing it is normal in color when passed, but on standing in contact with the air is blackened.

Test for Melanin.—To the urine add a few drops of bromin-water. A yellow precipitate forms which gradually blackens on standing.

Sodium nitroprussid added to the urine after it has been rendered alkaline with potassium hydroxid, and then made acid with acetic acid, after the addition of the sodium nitroprussid, gives a deep-blue color. This test is said not to be characteristic for melanin, but it will be found that in urines giving the test with bromin-water this reaction will be usually given.

Detection of Lead in the Urine.—In many cases the crucial diagnostic test in patients suspected to be suffering from plumbism is the detection of lead in the urine. This operation requires a large amount of the urine, and care must be taken

to see that the reagents employed are free from the metal. This may be proved by making an experiment, using the same quantities of the reagents with a sample of normal urine.

One thousand to 2000 c.c. of the urine are taken and made acid with 50 c.c. of concentrated nitric acid, and evaporated to about 100 c.c. in a large evaporating-dish. When the urine is evaporated to this bulk, it is transferred to a small evaporating-dish, and further concentrated over a free flame. The evaporation should be performed in a hood or in some place where the nitric acid fumes will not cause annoyance. When the urine is reduced to a thick paste the heat is increased. The mass deflagrates, and will usually leave a white residue at the end of the deflagration. If the mass be not white, the dish is allowed to cool, is moistened with a little nitric acid, and again heated. When the residue is quite white the dish is allowed to cool, and 20 c.c. of a 10 per cent. solution of acetic acid are added and the mixture boiled. The contents of the dish are filtered. The filtrate contains all the lead in the form of lead acetate. The perfectly clear and colorless filtrate is then divided into two portions in test-tubes, and examined for lead. To one portion is added 1 c.c. of a fresh solution of hydrogen sulfid. Should lead be present, the solution will be darkened owing to the formation of lead sulfid. This darkening will best be seen on looking down the mouth of the tube, so as to obtain as great a column of liquid as possible. For comparison, the other

tube which has not been treated with the sulfid may be used.

Into the other tube is dropped a crystal of potassium iodid the size of a mustard-seed. A yellow halo around the crystal will indicate the presence of lead. This will best be seen by placing the tube on a sheet of white paper.

Mercury.—Mercury is always present in the urine of patients undergoing mercurial treatment, and it is of importance in insurance work to be able to detect this element where it is suspected that the applicant has recently been under the influence of the drug. In cases of ptyalism also it will be of value to ascertain whether the mercury is being properly excreted.

Two hundred c.c. of the urine are boiled with 10 grams of zinc dust for ten minutes. The mercury is precipitated on the zinc and amalgamates with it. The zinc dust is filtered off, thoroughly washed with hot water, and dried at a low temperature, preferably in a water-oven at 100° C.

The zinc dust is introduced into a long and narrow test-tube, which should be perfectly dried before use. The zinc is shaken well down to the bottom of the tube, and the tube constricted slightly below the middle in the flame of a Bunsen burner, as shown in Fig. 33. The constriction is plugged very loosely with a small bit of asbestos wool, and on this wool is placed a crystal of iodine as large as a pin's head. The zinc dust is now heated strongly, and the iodine volatilized by bringing the constriction in the flame for an instant. The mercury is driven from

its combination with the zinc, and in passing through the constriction unites with the iodine to form mercuric iodid, which appears in the upper part of the tube in the form of a bright-red sublimate. This reaction when properly performed will show the minutest trace of mercury in the fluid to be tested.

Urinary Sediments, and Sedimentation.—The sedimentation of urine is accomplished in a tall



FIG. 33.—Tube used in testing for mercury.

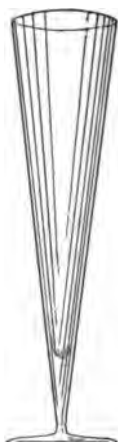


FIG. 34.—Cone-shaped cylinder.

cylinder, which is preferably in the form of an inverted cone (Fig. 34), and which should be provided with a cover to protect the urine from dust, which will enter the urine, fall to the bottom, and complicate the microscopic examination of the fluid. The urine should be allowed to stand at

least twenty-four hours before examination, and at the end of this time the sediment should be drawn off by means of a pipet. The sediment is transferred to a microscope slide and covered with a cover-slip, avoiding pressure. When casts are to be sought for, it is advisable to stain the preparation with methylene-blue. This is done by placing a drop of the staining-fluid at the edge of the cover-glass, and allowing it to diffuse in. The slide should first be examined with a low power, and any suspicious areas further investigated with a high power. The fine details of casts can hardly be seen except by using a magnification of from 300 to 450 diameters.

Should a thorough examination of the sediment be required shortly after the urine is voided, it will be necessary to resort to centrifuging.

The Centrifuge.—This instrument has come into very general use in the examination of urinary sediments, and will be found to give much information which the older method of sedimentation does not give. It has the further advantage that an examination can be made immediately after the urine has been passed, and therefore, any change which could take place in the structure of the sediment by reason of standing in contact with the urine in the air will be avoided. This is of great value where a search is being made for casts or blood-corpuscles, as after standing a few hours in the urine their appearance is much altered. There are many kinds of centrifuges designed for urine work. Some are driven by hand, others by water

power, and others again by electricity. The most satisfactory one which has been designed for hand power is that of Bausch & Lomb, of Rochester. This instrument is portable, and has the advantage of great mechanical simplicity. It is, moreover, easy to reach a high speed with it with the exercise



FIG. 35.—The Purdy electric centrifuge.

of comparatively little energy. It is also adapted for use in the examination of blood by means of the hematokrit. With water-driven centrifuges it is difficult to reach a sufficiently high speed with the water-pressure obtained in towns in this country. When they are allowed to run for a correspondingly great length of time they are very satisfactory.

The most convenient electric centrifuge is that designed by Purdy, of Chicago. This machine is built to be run with alternating or direct current at any voltage that may be desired. It is, of course, necessary in ordering a machine to state the kind

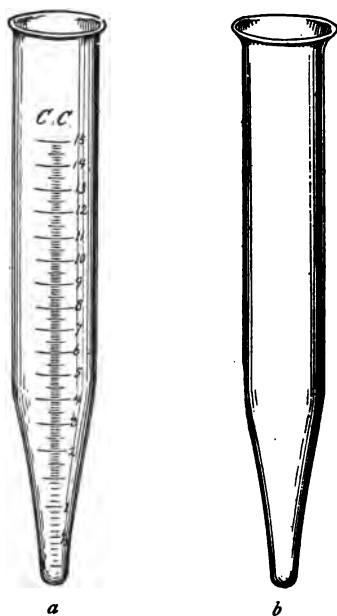


FIG. 36.—Tubes for the Purdy centrifuge : *a*, percentage tube ; *b*, sediment tube.

of current and the voltage required. A very convenient rheostat is provided, by means of which any desired speed may be obtained. There is also furnished with the later instruments a speed-counter which records the speed at which the instrument is

running. All electric centrifuges have the disadvantage that they require a certain amount of care in handling and adjustment. They also do not work well if kept in the rather trying atmosphere of the laboratory. On the other hand, they are by far the most convenient instruments for the pur-

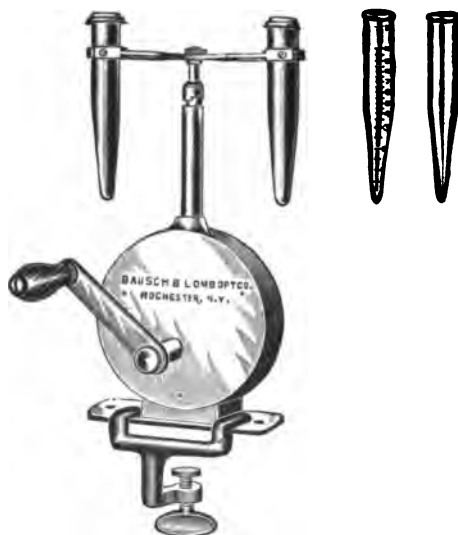


FIG. 37.—The Bausch & Lomb spiral-gear urinary centrifuge with tubes (one-fourth actual size).

pose, and in fact where many examinations of urinary sediments have to be made in the course of the day are the only machines for the purpose.

It has been proposed to use the centrifuge for the estimation of those constituents of the urine which can be separated out in the form of insoluble precipitates. To this class belong albumin, the chlorids

precipitated with silver nitrate, the phosphates with magnesia mixture, etc. This is scarcely possible except where the conditions as to speed and time of centrifuging, and temperature, are very carefully attended to. It has been found that comparatively small alterations in the speed of centrifuging have a relatively large influence in the bulk of the precipitate. Hence this method can at its

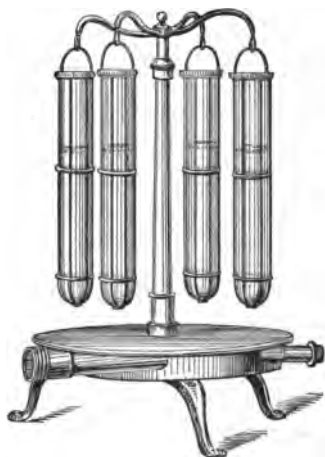


FIG. 38.—Water-power centrifuge.

best yield a very rough approximation of the real amount of the substance precipitated. As a clinical method for comparative work, however, it is not without value.

The Inorganic Sediments of the Urine.—Those inorganic sediments which occur in the urine are: The phosphates of ammonium, sodium, calcium, and magnesium, phosphates of calcium and

magnesium, and calcium carbonate. These sediments occur only in alkaline urine, while in acid urine the sulfate of calcium is rarely found.

The common organic constituents of urine sediments are: Uric acid, sodium and ammonium urates, calcium oxalate, and more rarely hippuric acid, cystin, tyrosin, and leucin. In alkaline urine ammonium urate is not an uncommon sediment. By



FIG. 39.—Triple-phosphate crystals (Ogden).

far the most commonly occurring sediment in alkaline urine is the triple phosphate of sodium, ammonium, and magnesium. This frequently makes up the major part of the stone or calculus following a cystitis.

The phosphates occur both in the amorphous and crystalline conditions. The crystalline forms of the phosphates are again subdivided into two forms, depending on the way in which the crystals separate

out. The first or feathery form is seen as branching, complicated crystals not unlike the frost-figures on glass. The second form are well-defined rhombohedral crystals with two of the opposite ends truncated. These are the familiar coffin-lid-shaped crystals. They are usually of large size. Both the feathery and rhombohedral forms may occur in the same sample of urine. There is no specific difference between them, the change in form being simply due to crystallization. They disappear on rendering the urine acid with hydrochloric acid.

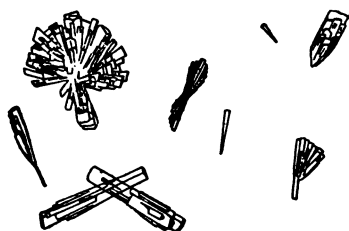


FIG. 40.—Acid calcium phosphate crystals (Ogden).

Simple phosphates of calcium and magnesium are of frequent occurrence in the urine. They are difficult to distinguish with the microscope. They form radiating bundles of stout prisms.

Calcium carbonate occurs but rarely in the sediment from urine. Should it be found that the sediment on being collected on the filter effervesced with hydrochloric acid, it would be fair presumptive evidence that the sediment contained calcium carbonate. The form which calcium carbonate assumes in the urine is small rhomboidal prisms.

Organic Sediments.—Uric Acid.—The most frequently occurring sediment in the urine is uric acid. The compound will be found in many forms, and is usually found in highly acid urines which have stood for some time. It is the sediment which is familiarly known as cayenne-pepper grains. It

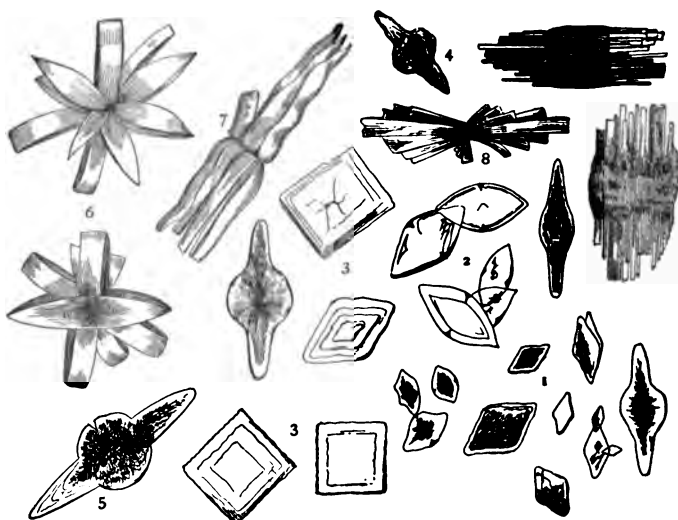


FIG. 41.—Forms of uric acid: 1, rhombic plates; 2, whetstone forms; 3, 3, quadrate forms; 4, 5, prolonged into points; 6, 8, rosetts; 7, pointed bundles; 9, barrel forms precipitated by adding hydrochloric acid to urine (Ogden).

is largely increased with a nitrogenous diet, and will also be seen in the urines of high specific gravity in cases of fever. It has the property of forming solid solutions with the coloring-matters of the urine, and of thus being precipitated in the urine in a highly colored condition. Its most usual

form is that of lozenge-shaped crystals which do not have a rectilinear outline. Often the narrower ends of the crystals converge suddenly to a point and become almost spiculated. This is well shown in Fig. 41. In many cases the crystals will be so thinned as to form a cross or many-pointed star. They are distinguished by their ready solubility in weak caustic alkalies.

Urates.—The urates should be examined closely, for they often simulate granular casts. The urates occur in the amorphous and crystalline conditions. The former are the more common. Urate of sodium

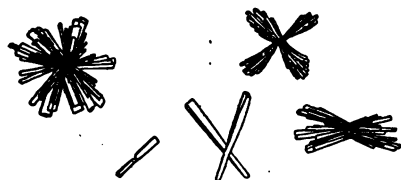


FIG. 42.—Acid sodium urate crystals (Ogden).

forms the *sedimentum lateritum* of morning urine. This is deposited from the urine as a fine, brick-dust-colored precipitate, which is amorphous. It is the acid sodium salt of uric acid, and disappears on making the urine strongly alkaline. Under the microscope this precipitate will be found to consist of exceedingly fine granules, of which no structure can be seen. The crystalline form of sodium urate is not characteristic.

Calcium oxalate occurs frequently in normal urine and assumes a variety of forms. The most characteristic shapes are the dumb-bell and envelope

forms. The former consist, as their name implies, of a bar with bulbous ends. The latter are really flattened octahedra, the edges of the crystal giving the peculiar envelope outline. They are insoluble in acetic acid, but are readily soluble in hydrochloric acid. The presence of oxalates in excess is often accompanied by nervous symptoms. Oxalates also give rise in the bladder and kidneys to small calculi,



FIG. 43.—Various forms of calcium oxalate crystals (Ogden).

which are often spiculated. Their passage through the ureters is a frequent cause of renal colic.

Cystin.—Cystin is an exceedingly rare deposit in the urine. It is curious in some cases of cystinuria in almost completely replacing the uric acid. It is found more often in calculi. It is readily soluble in ammonia, and crystallizes on the evaporation of the solvent in beautiful small hexagonal plates.

Creatinin is rare. It is said to be produced in increased quantities in acute parenchymatous nephritis. It is usually seen as small radiating bundles which tend to become hexagonal in outline.

Leucin and Tyrosin.—These crystals are seldom seen, but are important, because they occur in the urine of patients suffering from acute yellow atrophy of the liver, or in phosphorus-poisoning. They are

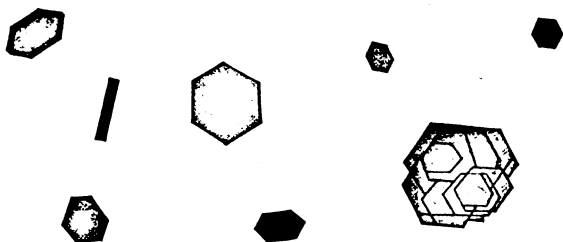


FIG. 44.—Cystin crystals (Ogden).

also occasionally seen in the urine of yellow fever patients. Leucin occurs as brownish-yellow globules. Tyrosin is found in sheaf-like bundles of needles.

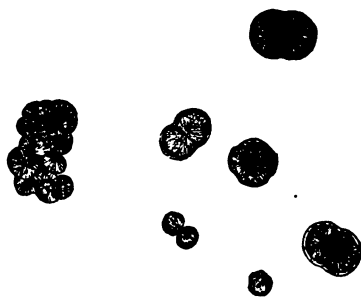


FIG. 45.—Leucin crystals (Ogden).

The Organized Structures of the Urinary Sediment.—The principal structures found in the urine are epithelial cells from various parts of the

urinary tract, blood-corpuscles, pus-cells, and casts. Besides these, one may have multicellular structures from the kidneys or bladder, and parts of morbid growths in these organs, and lastly micro-organisms which have been taking part in infective processes

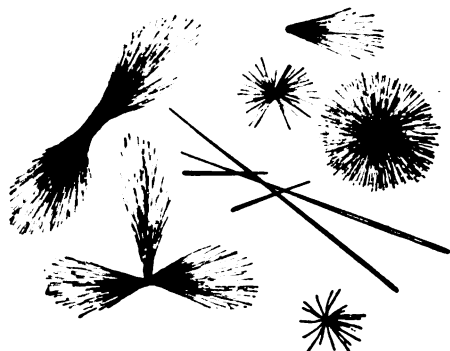


FIG. 46.—Tyrosin crystals (Ogden).

through the urinary tract or have been caught in the urine after it has been passed.

Epithelial Cells.—In every normal urine on careful examination will be found a certain number of cells derived from different portions of the genito-urinary tract. These cells differ widely in size and shape, and a certain amount of reliance may be placed on the distinguishing features when a sufficient number of the cells of like form are found.

This is especially the case where the pathological evidence is supported by clinical symptoms.

It must be remembered that a positive opinion cannot be formed as to the exact region whence the

cells come, as the changes which occur in the cells in a fluid like the urine are very great indeed. They cannot, therefore be expected to appear as

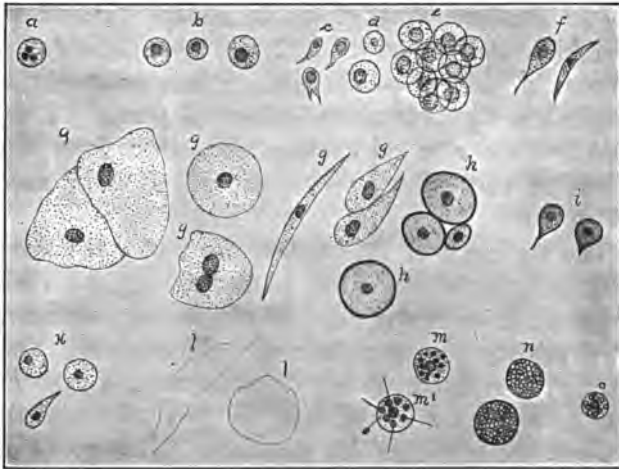


FIG. 47.—Epithelium from various parts of the urinary tract: *a*, leucocyte (for comparison); *b*, renal cells; *c*, superficial pelvic cells; *d*, deep pelvic cells; *e*, cells from calices; *f*, cells from ureter; *g*, *g*, *g*, squamous epithelium from the bladder; *h*, *h*, neck-of-bladder cells; *i*, epithelium from prostatic urethra; *k*, urethral cells; *l*, *l*, scaly epithelium; *m*, *m'*, cells from seminal passages; *n*, compound granule cells; *o*, fatty renal cell (Ogden).

they do in their normal position in the organ or tissue itself.

The epithelia from the **uriniferous tubules** are small and simulate pus-corpuscles closely. They are, as a rule, difficult to distinguish from the latter, but are, on the whole, somewhat larger in size.

The cells from the pelvis of the **kidney** are large, usually flattened and irregular in shape. They often present irregular ameba-like processes, which are somewhat characteristic.

The **ureteral epithelium** is always present in small amount in the urine, larger quantities being present in cases of renal colic. These cells are distinguished by their nearly spherical shape. They usually appear in association with cells from the pelvis of the kidney when the affection is renal colic.

The epithelia of the **bladder** are by far the most frequently occurring form in the urine, and their appearance is often of no pathological significance. They are the largest cells in the urinary tract. The number is enormously increased in cases of cystitis, due to whatever cause, and in this affection are always accompanied by pus-cells. The epithelia from the superficial layer of the bladder are flat, large, and highly irregular in outline. Several epithelia are often clustered together to form masses of cells.

The cuboidal and columnar epithelium from the deeper layers of the wall are found only in pathological conditions. They are smaller than the cells from the superficial layer and are more regular in outline. The columnar cells will be found to be more or less spindle-shaped. The spindle-cells occur only where the lesion is a deep one in the bladder-wall.

Urethral Epithelium.—These cells are commonly found in inflammations of the lower urinary tract.

They differ widely in their form, owing to the great number of epithelia found from the opening of the bladder to the meatus urinarius. Hence great reliance cannot be placed on a microscopic examination to detect their origin. The occurrence of pus-cells with gonococci, in conjunction with a large number of epithelial cells, will lead one to suspect that the latter have their origin in the urethra, and not at a point higher up.

Vaginal Epithelium.—These cells occur in the urine of females, especially in cases in which an acute or chronic vaginitis exists. They may usually be recognized by their large size, and they contain as a rule both cocci and bacteria. They are frequently found in aggregates of from two to five cells.

Uterine Epithelium.—This form of cell is distinguished by the delicate cilia which are so characteristic. Even when the cilia are broken off by contact with the urine, the remnants of them may be seen as slight projecting points on one end of a somewhat prism-like cell.

Renal Casts.—In all cases of disease of the kidney, and particularly in those which have to do with an affection of the secreting epithelium, there will be found certain organized elements in the urinary sediment which are known as tube-casts. These are in the form of cylinders, which may be either approximately straight or convoluted, and correspond in size to about that of the uriniferous tubules. They may have as their basis either an insoluble albuminous material or amyloid substance, or urates or other salts.

The method of their formation is not known. It has been suggested that they are derived from the coagulation of the albumin and globulin directly from the blood, as a sequence of pathological change in the secreting epithelium itself, or that the cast is a portion of the protoplasm of the epithelium which has been excreted and solidified.

The number of casts present in the urinary sediment will vary widely with the case and the time at which the urine has been taken. In some cases they are so plentiful as to be found without difficulty, while in others it may be only after the most careful examination of several slides and repeated centrifuging that the elements will be made out.

Care must also be taken not to confuse the casts with substances introduced from without into the urine, and for this reason the urine during sedimentation should be carefully covered, and all sides and cover-slips should be as free from lint and dust as possible.

The commonest forms of casts are: 1. Hyaline casts; 2. Granular casts; 3. Amyloid casts; 4. Epithelial casts; 5. Blood-casts; 6. Fatty casts; 7. Pus-casts; 8. Urate-casts; 9. Bacterial casts.

Hyaline Casts.—The nature of the ground-substance of these casts is unknown. They are the most difficult of all casts to detect in the urine on account of their refractive index being nearly the same as that of the fluid in which they float. It is only, therefore, by careful observation and focussing that they are detected. It is often well in looking

for these and other casts, if they have not previously been stained, to shade the mirror of the microscope with the hand, as the outline of the casts will be more clearly defined in the darkened field. The hyaline casts are cylindrical, usually long, almost colorless. As a rule, they are an indication of a chronic inflammation of the kidney, but they may also be found in other chronic diseases of that organ. They are common in the chronic interstitial and parenchymatous nephritides, in amyloid disease, and in passive hyperemia.

Granular Casts.—Granular casts are found in all diseases of the kidney, and are to be distinguished by the finely granular quality of the surface of the cast. In the brownish granular cast the granulation is evidently the result of the disintegration of blood-cells. In the colorless varieties the granulation is due to small fragments of cells which have affixed themselves to the surface of the cast.

Amyloid Casts.—These casts are more highly refracting than the former. They are large in size, and may often be seen with finely corrugated surfaces. These casts not infrequently present the amyloid reaction with iodine in potassium iodide, and with methyl-violet. They are found in the later stages of nearly all diseases of the kidneys, especially those associated with amyloid change. They are invariably of serious prognostic significance.

Epithelial Casts.—These are the most characteristic of all casts found in the urinary sediment, and are distinguished by the epithelial cells which cover

the surface of the cast. They usually result from acute inflammatory processes, where an active desquamation of the cells of the uriniferous tubules is taking place.

Blood-casts.—Casts consisting of masses of agglutinated red blood-cells occur in hemorrhagic conditions of the kidney. According to the condition of the blood at the time of the segregation of the corpuscles, and also the amount of diffusion of blood-pigment into the urine which has taken place, these casts will be pale yellow or colorless.

The blood-casts are usually associated with hematuria or hemoglobinuria, although cases are often found in which the appearance of blood-casts is not accompanied by other signs of blood in the urine.

Fatty Casts.—These structures are exceedingly characteristic, and are met with as colorless cylinders, of which the surface is studded with highly refractile oil-globules. As their name would indicate, they are closely associated with a fatty degeneration of the organ, and are usually met with in the later stages of chronic parenchymatous and acute nephritides.

Urate-casts are those in which the surface is coated with a deposit of amorphous urates. The urate usually seen is the ammonium compound.

URINARY DIAGNOSIS.

The diseases in which an examination of the urine will be of value may be divided into two classes:

1. Affections of the kidneys. These are the

renal hyperemias, acute and passive; chronic interstitial nephritis; amyloid disease of the kidney; cystic disease of that organ, both aseptic and purulent; infection of the renal parenchyma; and malignant disease.

2. Affections of the ureters.

3. Affections of the bladder. These are the cystitides, traumatic and infective; calculi; and disease due to malignant growths.

4. Disease of the urethra. This is usually of an infective character.

Affections of the Kidney.—Acute Hyperemia.

—Acute hyperemia may be due to any cause raising the general arterial tension, and is often found in the albuminuria accompanying the hyperpyrexia of scarlet fever, erysipelas, acute rheumatism, and diseases of this class. It is found as the result of long-continued and severe strain, both mental and physical.

The urine may contain blood, often only in traces, and albumin will almost invariably be present. Casts may or may not appear. They are usually of the hyaline variety. The quantity of urine voided in the twenty-four hours may be slightly increased. In hyperemia due to venous congestion the urine is usually dark in color. The specific gravity will also be raised, and this condition of things will also be accompanied by the other signs of venous stasis.

Chronic Interstitial Nephritis.—This condition, which is also known as chronic renal cirrhosis, or chronic Bright's disease, is perhaps the commonest

of renal affections. It is a disease of long duration, and may not be detected till an insurance examination or other adventitious circumstance renders the examination of the urine necessary. General fibrous interstitial change may accompany it, and this may be the result of long-continued alcoholism or exposure. The urine is invariably increased in quantity, is of light color, and somewhat low specific gravity. It will be found that the total amount of urea, as estimated in a sample of the whole amount of urine passed from day to day, is decreased. This, with a persistent low specific gravity, increase in quantity, and a small but ever-present amount of albumin, will point to this affection. A careful examination will often discover fibrous change in other organs.

Acute Parenchymatous Nephritis.—This is usually termed acute Bright's disease. The urinary features are well marked. These are decrease in quantity, increase in specific gravity, decrease in the total solids, dark color of the fluid, increase in the amount of sediment, the comparatively large amount of albumin, and the character of the casts. From the first, the quantity of urine is diminished, and in extreme cases will amount almost to complete suppression. The specific gravity ranges from 1025 to 1030. The urea, which may be above normal in any one sample examined, will be found to be greatly decreased when the amount for the twenty-four hours is taken into account.

The percentage of albumin is characteristic in being large. The usual quantity, as measured by

Esbach's albuminimeter, is 0.5–1.0 per cent. The progressive diminution of the albumin may be taken as a favorable diagnostic sign. Blood is often present in the urine, and renders the immediate prognosis unfavorable. The microscopic examination will reveal a large number of casts. These may be epithelial, and may have blood-corpuscles or leukocytes adhering to them.

Chronic Parenchymatous Nephritis.—The chief characteristics of this disease are decrease in the amount of urine passed per diem, decrease in specific gravity, and variability in color. The quantity of albumin is large, exceeding that of any other disease of the kidney. The total solids are decreased, the urea falling off markedly in quantity. The sediment is characteristic. The casts, which are present in comparatively large numbers, are hyaline, fatty, and granular. Fatty casts denote a case of long standing. Hyaline casts are somewhat indicative of recent disease of the organ. Granular casts will be found in all stages of the disease. The granulation increases as the disease becomes chronic. The presence of leukocytes is of frequent occurrence.

Amyloid Disease of the Kidney.—This condition is not of such frequent occurrence as the other diseases to which the kidney is subject; but as the correct diagnosis of this affection is absolutely necessary for the proper treatment, it is of importance that it should be recognized as early as possible.

The characteristic features of the urine are increase in volume, a considerable quantity of albumin, and

a fair number of casts of large size. These casts are of the amyloid variety, and are completely characteristic of this condition. They may be recognized by their yielding the usual amyloid reactions employed in microscopy.

If to a portion of the sediment a weak solution of iodine in potassium iodide be added, the color of the casts, which was originally a pale yellow, will change to a brownish red, which on the addition of sulfuric acid will turn blue. Methyl-violet may also be used. This stains amyloid tissue a pale green.

Infective Disease of the Kidney.—With the exception of cases of nephritis, which occur as the sequelæ of infective cystitis and ureteritis, the common disease of the kidney due to micro-organisms is renal tuberculosis. The disease may occur throughout the parenchyma, or be localized more or less in spots. An examination of the urine cannot differentiate between these two forms. The general diagnosis of a renal tuberculosis rests on the discovery of a nephritis associated with the detection of the *Bacillus tuberculosis* in the urine. In order to do this the urine must be centrifuged at the highest possible speed, and where possible the sediment of several centrifugings collected in one tube and submitted to renewed sedimentation. As the bacilli are never present in very large numbers, the greater the amount of urine concentrated in this way the greater the chances of success. After the sediment is collected it is transferred to a slide, dried at a gentle heat, and stained with the Ziehl-

Niehlssen carbol-fuchsin in the ordinary way. The counter-staining with methylene-blue may be omitted. It will usually be found that a considerable number of slides will be examined before the organism is found, so that failure to detect the micro-organism in the first half-dozen slides does not necessarily imply that further search will be fruitless. The other features of the urine are those of a pyuria, viz., the presence of a large quantity of albumin with the occurrence of pus-cells. Blood often accompanies the pus.

Malignant Disease of the Kidney.—This may be carcinomatous, sarcomatous, or lymphadenomatous. The examination of the urine is, as a rule, of no very great diagnostic importance. The urine may exhibit the characters of an irritation of the kidneys. Blood is frequently found, and may be intermittent. The cells of the malignant growth are seldom recognized in the urine.

Uremia is often the fatal termination of many diseases of the kidneys where the organ fails to eliminate the waste products, and the skin does not take up the vicarious function with sufficient rapidity to allow the kidneys to recover. It is a toxemia, but to what specific agent the toxic action is due is not known.

The most distinctive feature of the urine is the deficiency in the total solids, and in particular the urea, which may be reduced to one-tenth the normal amount. The volume and the specific gravity may both be decreased simultaneously. The former may be said to be always below normal, in most cases

markedly so. The specific gravity is not characteristic. Albuminuria is practically always present, as are casts. The amount of albumin is often large, sometimes exceeding 3 per cent. by weight.

Diabetes Mellitus.—The clinical feature of this disease is, of course, the presence of glucose in the urine. The amount of this may be so small as to require care in its detection. This is often the case in the glycosuria of persons of stout habit. As a rule, however, the amount is sufficient to give a marked reaction with Fehling's solution. The polyuria is always an important symptom, and varies from slightly over normal to the enormous quantity of 25 liters in the twenty-four hours. The reaction of the urine is acid. The urine has a sweet smell, due to the presence of ethereal salts in solution. Acetone, aceto-acetic acid, and oxybutyric acid are present in the later stages of the disease. Acetone existing alone is said to be of favorable prognostic significance, while the presence of aceto-acetic acid and β -oxybutyric acid is a sign of a more unfavorable termination of the malady. Aceto-acetic acid and β -oxybutyric acid are said to be inseparably connected with diabetic coma. A certain amount of albumin is often present, due probably to the irritation of the secreting cells of the kidney by the glucose. The urine is often of a frothy character, so much so that the detection of acetone is annoyingly complicated by the froth from the urine which is distilled, filling a flask of almost any size.

Vesical Calculus.—The urine in cases of stone

in the bladder presents the characters of a cystitis, which may or may not be complicated by infective symptoms. The latter is often the case where the patient has been repeatedly catheterized and infection from without has taken place. The quantity of urine is normal, and may be clear. The reaction may be either acid or alkaline. In advanced cases the urine may display a highly alkaline character, due to the ammoniacal decomposition of the urea in the bladder. At the same time the urine will be turbid from the precipitation of triple phosphates. According as the bladder-wall is affected by the foreign body, the quantity of pus will vary, as will also the amount of blood. Large numbers of cells from the denuded mucosa of the bladder will be seen.

The Examination of Calculi.—The calculi found in the bladder will consist chiefly of uric acid, urates, oxalate of calcium, simple phosphates of calcium and magnesium, phosphates of ammonium, magnesium, and calcium, and more rarely of cystin. Practically all calculi will respond to the reactions for uric acid, because the reactions usually employed are so delicate and the calculus has remained in the urine so long a time as to be completely impregnated with this compound. It must also be remembered that calculi are, as a rule, never strictly homogeneous. The nucleus may consist of a shred of mucus or tissue, or often of a fragment of a crystal of uric acid. The succeeding layers will consist of the same substance or other substances which happen to be the least soluble in the urine at the time, and

hence have been precipitated. Thus it not infrequently happens that a careful examination of a calculus will reveal the presence of from two to six substances forming the structure.

Uric Acid.—Uric acid may be detected by dissolving a fragment of the calculus in dilute potassium hydroxid, and adding hydrochloric acid. The crystals precipitated will conform to one of the shapes seen in Fig. 41, page 152.

If a small particle of the calculus be moistened with nitric acid and evaporated at a gentle heat in a porcelain capsule, a yellowish residue is left if uric acid be present. On exposing this residue to the fumes of ammonia, or adding a drop of ammonium hydroxid, a splendid purple color will be obtained. This test is given by almost any calculus, owing to its sensitiveness.

Uric acid, ignited on platinum foil, chars and eventually disappears.

Calcium and Magnesium Phosphates.—These salts give a white residue when ignited on platinum foil. Calcium may be detected by moistening with strong hydrochloric acid after ignition, and exposing a particle of the moist residue to the Bunsen flame on a platinum wire. The presence of the calcium will be shown by the orange color imparted to the flame.

Magnesium is best detected by moistening the residue with a drop of cobalt nitrate solution and igniting. The salts of magnesium treated in this way leave a flesh-colored residue.

Phosphoric Acid is best detected by heating the calculus with some strong potassium hydroxid in a

test-tube, and adding a piece of magnesium ribbon. The presence of phosphoric acid is made evident by the characteristic smell of phosphin. If a fragment of the calculus be dissolved in strong nitric acid and ammonium molybdate solution be added, a bright-yellow precipitate of ammonium phosphomolybdate forms in the presence of phosphoric acid.

Triple Phosphates.—In this class of calculi the ammonia may be detected by moistening the calculus with potassium hydroxid, and heating gently. The ammonia evolved may be detected by the smell, and the reaction may be further confirmed by the alkaline reaction of the gas on a piece of moistened red litmus-paper.

Oxalates.—Oxalates leave a grayish-white residue by heating on platinum foils which effervesces with dilute hydrochloric acid. The fragment which has not been ignited does not effervesce.

Cystin.—The calculi of this substance are rare, but are readily recognized by dissolving in ammonia. The solution on evaporation deposits crystalline plates which are exceedingly characteristic. The substance is completely volatile on ignition.

EXAMINATION OF THE GASTRIC CONTENTS.

IN examination of the gastric contents it is important to start under definite conditions. For this reason it is inadvisable to use the vomit or the gastric contents withdrawn by the stomach-tube except after a test-breakfast. The character of the contents varies widely during the day. Digestion is furthest advanced an hour after a meal, although the secretion of the gastric fluid commences immediately on the reception of the food by the stomach, or perhaps somewhat earlier, from reflex nervous stimulation. The amount secreted in the twenty-four hours is about 13 liters.

The test-breakfast consists of 35 grams of dry unbuttered bread, and 300 c.c. of warm water or weak unsweetened tea, without milk. One hour after the ingestion of the food the contents of the stomach are withdrawn by the stomach-siphon. This apparatus consists of a long red-rubber tube, 7 mm. in diameter, having near the closed end two or three small openings about 5 mm. in diameter. The tube is warmed gently, and introduced into the back of the pharynx, and held there till the patient makes an effort to swallow. It is then gently guided down till the end is well within the stomach. It is often useful to have a mark on the tube to indicate the length necessary to enter the stomach. If too much of the tubing

be within the gastric cavity, the curling of the tube interferes with the proper siphonage. If now gentle pressure be made on the abdominal wall, or the patient be induced to cough, the contents of the stomach will be expelled. The tube should be sufficiently long to have the free end below that within the stomach. Otherwise the tube will not act as a siphon. The contents should be received in a graduated glass.

If the test-breakfast be given in the proper quantity, the amount of fluid which will be obtained will be about 40 c.c. Any sediment which settles out should be examined with the microscope. The fluid should now be filtered and tested for hydrochloric acid and pepsin.

As a rule, the fluid filters easily. As removed from the stomach with the tube the normal quantity of hydrochloric acid is about 0.3 per cent. This appears to be the exact quantity necessary to check fermentative change. If less than this quantity be present, the juice rapidly undergoes fermentation.

The composition of normal gastric juice mixed with saliva was found by Schmidt to be:

Water	994.40
Solids	5.60
Organic substances	3.19
Sodium chlorid	1.46
Calcium chlorid	0.06
Potassium chlorid	0.55
Ammonium chlorid
Hydrochloric acid	0.20
Calcium phosphate	} 0.12
Magnesium phosphate	
Ferric phosphate	

From a diagnostic point of view, the important constituents of the fluid are free hydrochloric acid and the digestive ferments. Free hydrochloric acid always exists in the contents of the stomach in health, but may be entirely absent in certain nervous affections and in organic disease of the stomach, particularly in malignant growths attending the cardiac end.

Hydrochloric Acid.—The qualitative detection of hydrochloric acid may be accomplished with one of the anilin dyes or with Günzburg's reagent. Günzburg's test, which has been described in the section on the Gastric Juice, page 61, is best performed by dissolving up a small quantity of phloroglucin and of vanillin in 1 c.c. of alcohol, and adding to the mixture an equal quantity of the gastric filtrate. The mixture is placed in a porcelain basin, and evaporated over a water-bath. It is not advisable to heat with a free flame. In the presence of 0.05 per cent. of hydrochloric acid, a bright-red stain will be left, and with a larger quantity microscopic crystals will be seen. If the acid be absent, the residue will have a light-brown color.

Of the anilin dyes which have been proposed, Congo-red, benzopurpurin 6B, tropeolin oo, and dimethylamido-azobenzene, are the most used.

These dyes may be used in the form of solution, or more conveniently for clinical work in the form of test-papers. These may easily be made by soaking small strips of filter-paper or perfectly neutral unglazed paper in a strong solution of the indicator, and allowing them to dry in the air. Care must

naturally be taken to have the papers dry in an atmosphere where acid fumes are absent. The test-papers are not so delicate for indicating minute traces of the acids, but in this are perhaps to be preferred for bedside work, as the organic acids themselves will give indications of an acid reaction with solutions of indicators where the papers fail. Of the solutions, according to the author's experiments, the most sensitive is that of Congo-red. With it one is able to detect 4 parts in a million.

Tropeolin oo is also a delicate indicator of free acid. In the presence of acids the solution turns red.

Somewhat more sensitive than tropeolin oo is benzopurpurin 6B. The indicator sold under this name is not always the one which gives a sensitive reaction with acids, and therefore it may be necessary to obtain several samples before the right one is found.

In performing the tests for free acid in the stomach contents, it is only necessary to make up a dilute solution of the indicator, of about 0.2 gram in 50 c. c. of alcohol or water, and use 2 drops of this solution diluted with 10 c. c. of water. The stomach contents are then added drop by drop and the change of color observed.

Lactic Acid.—Lactic acid is produced in the gastric contents from the fermentation arising from the *Bacillus lacticus*. It may be detected by using Uffelmann's reagent. This is made by dissolving a small crystal of phenol in 10 c.c. of water, and adding a drop of a very dilute solution of ferric

chlorid. An amethyst-blue solution results. If now to this solution a portion of the gastric contents be added containing lactic acid, the blue color will be discharged, to be replaced by a faint lemon-yellow.

If, also, the filtrate from the stomach contents be added to a dilute solution of ferric chlorid, and lactic acid be present, the faint-yellow color will give place to a much deeper tint.

In both these tests it is better to shake a few cubic centimeters of the gastric filtrate with a few cubic centimeters of ether in a test-tube, and after evaporating the solution of lactic acid in ether, by allowing the ether to stand in a watch-glass, to dissolve the residue in a little water, and to use the solution so obtained for the Uffelmann and ferric chlorid tests.

Butyric Acid.—This acid is often present in the gastric contents in cases of marked dilatation of the stomach. It may be recognized in the ethereal extract by the characteristic odor of rancid butter.

The Digestive Ferments.—There are, unfortunately, no quantitative tests that may be easily carried out, by which the amount of pepsin, and therefore the digestive power of the gastric contents, may be quantitatively estimated. One can simply examine the fluid to see if the digestive power is still present. In order to do this, some compound whose change in digesting may be readily observed is taken. For this purpose either coagulated egg-albumin or fibrin may be used. The latter acts more satisfactorily, but the former is more conven-

ient for ordinary clinical work. With fibrin the digestion proceeds at room-temperature, while egg-albumin requires the temperature of the body—about 37° – 39° C.—for digestion. The fibrin used should be derived from ox-blood. A small bit of the washed fibrin should be placed in a test-tube and covered with the gastric juice. If digestion takes place, the fibrin swells up and finally disappears.

If egg-albumin be used, the uncoagulated white of egg is drawn up into small tubes made by drawing out ordinary quill tubing in the flame, and coagulating the albumin by passing the capillary tube containing it through the flame till the contents are white. This may be placed in a tube containing gastric juice and the whole placed in an incubator at 40° C. Another method consists in coagulating the albumin in the shell and cutting out cylinders, about 5 mm. in diameter, with a cork-border, or glass tube. These cylinders are cut thin in the form of circular slices, and are then placed in test-tubes containing the gastric juice.

Examination of the Vomit.—It may be necessary in some cases of suspected poisoning to examine the vomit. The same procedure for the clinical examination will be applicable as in dealing with a test-meal. It will be found that the fluid will be even more complex than that obtained where only bread and water have been given. The vomitus will contain larger amounts of organized matter, the result of the breaking down of food. Among the substances which will be encountered in the microscopic examination will be

muscle-fibers, crystals or globules of fat or of fatty acids, elastic fibers, connective tissue, starch-granules, and vegetable cells.

Diagnosis of Gastric Disease.—Acute Gastritis.—In this disease, which may or may not follow mechanical or chemical irritation of the stomach, the clinical features are well marked. Hydrochloric and lactic acids are often lacking at the outset, and the digestive power of the gastric juice will be found to be below normal. Bile-pigment and the biliary salts will be sometimes found in the vomit, and will be recognized by the appropriate tests.

Chronic Gastritis.—The vomit in this disease is thin, and in cases associated with dilatation of the stomach will be excessively so. Hydrochloric acid will usually be found to be present, although in rare cases the contents of the stomach have been alkaline in reaction.

Simple Gastritis.—In simple gastritis the test-breakfast is not followed by increased acidity, and the proportion of hydrochloric acid is usually below normal. The pepsin is also proportionally decreased. In acid gastritis the hydrochloric acid is above normal, and may be increased over 100 per cent.

In **mucous catarrh of the stomach** the amount of acid falls very considerably, and in a large percentage of cases is entirely absent.

In **atrophic gastritis** the contents of the stomach are free from hydrochloric acid, pepsin, and the milk-curdling ferment.

Chronic Ulcer of the Stomach.—In this disease the clinical features which are of importance are the excess of hydrochloric acid and the presence of blood. The hyperacidity of the gastric juice is usually very marked in gastric ulcer, and may range from 0.4 to 0.6 per cent. As the disease progresses the hyperacidity diminishes. The blood may be in clots, or from contact with the hyperacid contents of the stomach may be changed to dark-brown, coffee-colored masses which consist chiefly of acid hematin.

Gastric Carcinoma.—The examination of the contents of the stomach in this most important disease may reveal more or less characteristic features. Hydrochloric acid may be said to be practically always absent. Cases in which it is found are rare. *Sarcinæ ventriculi* are often present in large numbers. Lactic acid may replace the hydrochloric acid, and hence may be found in relatively large quantity.

Gastric Dilatation.—The stomach contents in this disease show the general characters of enfeeblement of digestive power, and the loss of activity of the stomach-wall. The contents are, as a rule, dilute, and contain the remains of undigested food. The signs of long-continued fermentation are well marked. Yeast-cells and *sarcinæ* will be seen, and the amount of hydrochloric acid will vary within wide limits.

TABLES FOR URINARY DIAGNOSIS.

The following is a list of the diseases of the urogenital tract (those which are distinguishable through the urine are so marked); also a list of systemic diseases and special conditions causing definite changes in the urine.

The characteristic diagnostic signs in the urine, when present, are given. The list of diseases is complete, and therefore includes many pathological conditions which yield no characteristic signs in the urine. These are so designated. By consulting such a list the diagnostician may either from definite signs or by exclusion often arrive at a diagnosis.

While certain deviations from the normal occur in the urine under pathological conditions, and while these deviations are definite indices of such pathological condition, yet there are many deviations from the normal, the interpretation of which is quite obscure, and many pathological conditions, even of the urogenital tract, which yield no clue by the urine; moreover, several pathological conditions give the same urinary conditions, and are therefore indistinguishable from one another through this means. We must, therefore, as in all chemical and microscopical clinical investigations, consider our chemical and microscopical and physical examinations complementary one to the other.

I. DISEASES OF THE UROGENITAL TRACT.

1. **Malformations of the external genitalia** (no urinary changes).

2. **Affections of the external genitalia.**

Herpes and abrasions.

Chancres and chancroid.

Growths, benign and malignant.

(No urinary changes.)

3. Urethra.

<i>Name.</i>	Urethritis, acute, non-gonorrheal (urinary changes).
<i>Synonym.</i>	Inflammation of urethra.
<i>Color.</i>	Nothing characteristic.
<i>Odor.</i>	" "
<i>Quantity.</i>	" "
<i>Reaction.</i>	" "
<i>Spec. Grav.</i>	" "
<i>Albumin.</i>	" "
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Nothing characteristic.
<i>Sediment.</i>	" "
<i>Bacteriologic.</i>	Negative for gonococcus.
<i>Remark.</i>	This may occur (rarely) in the acute infectious diseases or as the result of masturbation. The discharge never reaches the thick, purulent condition seen in gonorrheal urethritis. May occur also as the result of injections or the ingestion of substances irritating to the mucous membrane.

<i>Name.</i>	Urethritis, acute, gonorrheal (urinary changes).
<i>Synonyms.</i>	Clap; tripper.
<i>Color.</i>	Variable.
<i>Odor.</i>	"
<i>Quantity.</i>	"
<i>Reaction.</i>	Acid or alkaline.
<i>Spec. Grav.</i>	Dependent upon the amount of sediment.
<i>Albumin.</i>	According to amount of pus.
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Normal.
<i>Sediment.</i>	Macroscopic: heavy, white. Microscopic: leukocytes; pus, according to stage of inflammation; red blood-cells, according to stage of inflammation.
<i>Bacteriologic.</i>	Gonococci, free and in leukocytes and pus.
	Urethritis, chronic (urinary changes).
<i>Synonym.</i>	Gleet.

<i>Color.</i>	Opaque, whitish.
<i>Odor.</i>	Heavy.
<i>Quantity.</i>	Variable.
<i>Reaction.</i>	Acid or alkaline.
<i>Spec. Grav.</i>	Variable.
<i>Albumin.</i>	Present according to amount of pus.
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Normal.
<i>Sediment.</i>	Macroscopic: thick, matted, white, with clear urine above. Microscopic: pus, mucus, débris; fibrin threads covered with pus and epithelial cells—Tripper-faden, or gonorrheal threads as they are called.
<i>Bacteriologic.</i>	Gonococci often found.
<i>Remark.</i>	No definite conclusion by the examination of the urine can be arrived at as to the locality in the urethra of the inflammation.

Chancre (urethral). The urine presents the characteristics of the *early stage* of a non-gonorrheal urethritis.

4. Prostate, Testicles, and Seminal or Spermatic Vesicles.

	Prostatitis.
<i>Synonym.</i>	Inflammation of prostate.
	Orchitis.
<i>Synonym.</i>	Inflammation of testicles.
	Spermatic or seminal vesicles.
	Inflammation of.
	In all these conditions the urinary changes are variable.
	Sediment may show spermatozoa; gonococci may be present.
	These are not infrequent primary, or more often secondary, seats of tuberculosis, in which case the tubercle bacillus may be found.
	In general the urine is that of chronic urethritis.

5. Bladder.

	Cystitis (urinary changes).
<i>Synonym.</i>	Inflammation of the bladder.
<i>Color.</i>	Opaque, muddy.
<i>Odor.</i>	Ammoniacal.
<i>Quantity.</i>	Normal. May be retention.
<i>Reaction.</i>	Acid or alkaline.
<i>Spec. Grav.</i>	Usually normal.
<i>Albumin.</i>	Usually present.
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Normal.
<i>Sediment.</i>	Macroscopic: in <i>acid cystitis</i> the sediment is more scant, more granular, and of a grayish color. In <i>alkaline cystitis</i> it is much more abundant, of a thick, matted character, very white, and consists largely of phosphates. No other condition presents in lately passed urine so characteristic a sediment.
	Microscopic: pus, leukocytes, red blood-corpuscles, separate and in clumps, fibrin in clumps, epithelium whole and mutilated, detritus.
<i>Bacteriologic.</i>	Myriads of micro-organisms; certain infections produce what is known as acid cystitis, others alkaline cystitis. Bacteriologic investigation is as yet very unsatisfactory.
<i>Remark.</i>	The only difference between the acute and the chronic cystitis is that noted in the different stages of any inflammation of the mucous membrane. A cystoscopic examination should always be made.
	Calculus (urinary changes).
<i>Synonyms.</i>	Vesical calculus and stone in the bladder. Urine normal at first, later similar to cystitis.
<i>Sediment.</i>	Macroscopic: resembles sediment of cystitis, but may be blood-stained, especially after straining at micturition or stool.

Microscopic: uric acid, oxalate of lime. A cystoscopic examination should always be made.

Growths (no characteristic urinary changes).
Synonyms. Vesical growths, neoplasms, tumors.
Remark. Practically no urinary changes except occasional paroxysms of hematuria. This, however, may be absent in some of the vesical growths. A cystoscopic examination should always be made.

Tuberculosis.
Synonyms. Vesical tuberculosis (urinary changes).
 Similar to cystitis, except that the urine is usually acid, and that the sediment contains the tubercle bacillus. A cystoscopic examination should always be made.

6. Ureter.

The affection which most commonly involves the ureter is tuberculosis, and this is always associated with a similar affection of the kidney either as a pyelitis or by the presence of miliary tubercles. There is nothing characteristic in the urine beyond the presence of the tubercle bacillus.

7. Kidney.

Name. **Hyperemia—active** (urinary changes).
Synonym. Active congestion.
Color. Dark.
Odor. Normal.
Quantity. At first increased, later diminished.
Reaction. Acid.
Spec. Grav. Normal.
Albumin. Present, small quantity.
Sugar. Absent.
Urea. Normal.
Sediment. Macroscopic: moderate in amount.
 Microscopic: epithelium, renal in type, leukocytes, red blood-cells, few in number, occasionally hyaline casts.

Name.	Hyperemia—passive (urinary changes).
Synonym.	Passive congestion.
Color.	Variable, may be dark or very pale.
Odor.	Normal, quantity diminished.
Reaction.	Acid.
Spec. Grav.	High—1020 to 1030, even more.
Albumin.	Trace.
Sugar.	Absent.
Urea.	Normal.
Sediment.	Macroscopic: considerable in quantity, flocculent. May be cellular. Microscopic: cylindroids, large quantity of epithelium, red blood-corpuscles. May be hyaline and blood-casts.
Remarks.	It is most important to distinguish between a true nephritis and active and passive congestion. <i>Active congestion</i> may occur in any of the febrile diseases; it may also be the result of the use of irritants, either injected or ingested. <i>Passive congestion</i> is due to cardiac or pleural diseases or any similar condition allowing of the stagnation of blood in the kidneys.
Name.	Pyelitis (characteristic urinary changes).
Synonym.	Term usually denotes pus <i>at the pelvis</i> of kidney, though etymologically it means simple purulent inflammation.
Color.	Muddy, opaque, dirty yellow.
Odor.	Ammoniacal.
Quantity.	May be increased.
Reaction.	Acid or alkaline.
Spec. Grav.	Usually reduced, dependent upon quantity of sediment.
Albumin.	Trace.
Sugar.	Absent.
Urea.	Normal.
Sediment.	Macroscopic: large quantity of debris, broken-down cells, mostly of white color. Microscopic: epithelium, red blood-cells occasionally seen, phosphates, both crystalline

and amorphous, in alkaline urine. Pus-cells in great quantity.

Bacteriologic. Myriads of bacteria.

Remarks. Catheterization of the ureters is now a safe and useful method not only of differentiating pyelitis from cystitis, but also of determining which kidney is the source of the inflammatory exudate. This method is available for both the male and female. See *Remark* under *Tuberculosis of Kidney*.

Name. **Nephritis, acute parenchymatous** (characteristic urinary changes).

Synonyms. Desquamative nephritis, tubal nephritis, diffuse nephritis, acute renal dropsy, acute albuminuria, acute Bright's disease, glomerular nephritis.

Color. Dark opaque. See *Hematuria*.

Odor. Nothing characteristic.

Quantity. Greatly diminished; may be suppressed (anuria).

Reaction. Acid or alkaline.

Spec. Grav. Usually high—1020 to 1026.

Albumin. Large quantity, half of 1 per cent. to 1 per cent.

Sugar. Absent.

Urea. Reduced.

Sediment. Macroscopic: abundant and granular. Microscopic: red and white blood-cells, casts of all varieties.

Name. **Nephritis, chronic parenchymatous** (characteristic urinary changes).

Synonyms. Tubal nephritis, diffuse nephritis, large white kidney of nephritis, chronic Bright's disease, chronic albuminuria.

Color. Muddy yellow, opaque.

Odor. Ammoniacal.

Quantity. Diminished.

Reaction. Acid or alkaline.

Spec. Grav. High—1020 to 1025.

<i>Albumin.</i>	Considerable quantity. Increases with advance of the disease and varies greatly during progress of same.
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Reduced.
<i>Sediment.</i>	Macroscopic: abundant, granular, and cellular. Microscopic: a few red and white blood-cells, casts of all varieties, much detritus, epithelium, whole and fragmented.

<i>Name.</i>	Nephritis, chronic interstitial (characteristic urinary changes).
<i>Synonyms.</i>	Granular kidney, contracting granular kidney, gouty kidney, fibrosis of kidney, cirrhosis of kidney, chronic Bright's disease.
<i>Color.</i>	Very pale yellow, transparent.
<i>Odor.</i>	Nothing characteristic.
<i>Quantity.</i>	Increased.
<i>Reaction.</i>	Usually acid, may be alkaline.
<i>Spec. Grav.</i>	Very low usually.
<i>Albumin.</i>	Mere trace.
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Reduced.
<i>Sediment.</i>	Macroscopic: very slight amount, flocculent. Microscopic: casts, scanty, hyaline, and granular, few epithelial cells, uric acid crystals, and blood-corpuscles.

<i>Name.</i>	Pyelonephritis (characteristic urinary changes).
<i>Synonyms.</i>	Surgical kidney, suppurative interstitial nephritis, acute interstitial nephritis.
<i>Color.</i>	Pale yellow.
<i>Odor.</i>	Ammoniacal.
<i>Quantity.</i>	Increased.
<i>Reaction.</i>	Usually acid, may be alkaline.
<i>Spec. Grav.</i>	Low.
<i>Albumin.</i>	Present in very large quantity.
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Reduced.

- Sediment.** **Macroscopic:** thick, white, matted layer; when flask is shaken sediment rises almost in mass. May be so thick that it plugs mouth of bottle in pouring out urine.
Microscopic: epithelium in large quantities, fragmented and whole, pus, red and white blood-corpuscles. Hyaline and pus-casts. Phosphates, amorphous and crystalline.
- Bacteriologic.** Myriads of bacteria.
- Name.** **Pyonephrosis.** See *Hydronephrosis*. (No characteristic urinary changes.)
- Sediment.** Pus, blood, mucus, epithelium.
- Bacteriologic.** Myriads of micro-organisms.
- Name.** **Hydronephrosis** (no characteristic urinary changes).
- Color.** Pale.
- Odor.** Nothing characteristic.
- Quantity.** May be greatly increased at one time or greatly diminished at another.
- Reaction.** Acid or alkaline.
- Spec. Grav.** Low.
- Albumin.** Absent or a trace.
- Sugar.** Absent.
- Urea.** Diminished.
- Sediment.** Macroscopic :
 Microscopic : epithelium, no casts.
- Name.** **Cystic Disease.** See *Hematuria and Chronic Interstitial Nephritis*. (No characteristic urinary changes.)
- Name.** **Infarct** (no characteristic urinary changes).
- Color.** Resembles that of hematuria and hemoglobinuria.
- Odor.** Nothing characteristic.
- Quantity.** Reduced.
- Reaction.** Usually alkaline, may be faintly acid.
- Spec. Grav.** High.
- Albumin.** May be present in large quantity, but appears suddenly, and may diminish or disappear as suddenly.

<i>Sugar.</i>	Absent.
<i>Urea.</i>	Normal.
<i>Sediment.</i>	Macroscopic: quantity variable. Microscopic: cylindroids, hyaline and epithelial casts, red blood-corpuscles, and a few leukocytes.
<i>Name.</i>	Calculus (urinary changes variable).
<i>Synonym.</i>	Stone in the kidney (nephrolithiasis).
<i>Color.</i>	Attacks of hematuria are characteristic, but there may be intervals of normal colored urine.
<i>Odor.</i>	Nothing characteristic.
<i>Quantity.</i>	Normal, or at times diminished; may be anuria.
<i>Reaction.</i>	Usually acid.
<i>Spec. Grav.</i>	May be increased.
<i>Albumin.</i>	Trace.
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Normal.
<i>Sediment.</i>	Macroscopic: may be abundant, usually granular. Microscopic: red blood-cells, especially after an attack of hematuria or renal colic; red blood-cells, epithelial cells, uric acid, and oxalate of lime crystals; if disease is of any duration, pus will appear in varying quantities.
<i>Name.</i>	Renal growths, benign or malignant (no characteristic urinary changes).
<i>Color.</i>	See <i>Hematuria</i> . This occurs in paroxysms.
<i>Odor.</i>	Nothing characteristic.
<i>Quantity.</i>	Normal.
<i>Reaction.</i>	Acid or alkaline.
<i>Spec. Grav.</i>	Varies with amount of sediment.
<i>Albumin.</i>	Trace.
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Normal or diminished.
<i>Sediment.</i>	Macroscopic: Microscopic: casts granular and hyaline. Much epithelial debris, red blood-cells, and clots.

<i>Name.</i>	Amyloid disease (urinary changes variable).
<i>Color.</i>	Transparent.
<i>Odor.</i>	Nothing characteristic.
<i>Quantity.</i>	Increased.
<i>Reaction.</i>	Acid or alkaline.
<i>Spec. Grav.</i>	Low.
<i>Albumin.</i>	Trace.
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Decreased.
<i>Sediment.</i>	Macroscopic : Microscopic : hyaline and waxy casts ; other casts may occur.
<i>Name.</i>	Tuberculosis (positive signs may occur in urine ; the disease may exist without these signs appearing).
<i>Color.</i>	Pale, opaque.
<i>Odor.</i>	Nothing characteristic.
<i>Quantity.</i>	May be increased or normal.
<i>Reaction.</i>	Acid or alkaline.
<i>Spec. Grav.</i>	Normal.
<i>Albumin.</i>	Trace.
<i>Sugar.</i>	Absent.
<i>Urea.</i>	Normal.
<i>Sediment.</i>	Macroscopic : Microscopic : pus, red blood-cells.
<i>Bacteriologic.</i>	The condition is determined positively by the detection of the tubercle bacillus or by positive results following animal inoculation. The tubercle bacillus must be differentiated by proper methods from the smegma bacillus.
<i>Remark.</i>	Tuberculosis usually takes the form of pyelitis. It must be remembered, however, that tuberculosis of the urogenital tract seldom remains in one locality, but usually extends throughout. An exception to this is found in the testicle, where tuberculosis is quite common and frequently overlooked. The tubes and ovaries should be mentioned as even more common seats, while the uterus is a very rare seat.

<i>Name.</i>	Nephroptosis.
<i>Synonyms.</i>	Movable kidney, floating kidney (palpable kidney [?]), ren mobilis, enteroptosis, visceroptosis, Glénard's disease, splanchnoptosis. (The four latter terms are employed when several or all the viscera are displaced.)
	The urine is normal, except during the paroxysms of pain known as Dietl's crises—during these the following characteristics are noted :
<i>Color.</i>	High colored.
<i>Odor.</i>	Nothing characteristic.
<i>Quantity.</i>	Variable.
<i>Reaction.</i>	Acid or alkaline.
<i>Spec. Grav.</i>	Variable.
<i>Albumin.</i>	Present or absent.
<i>Urea.</i>	?
<i>Sediment.</i>	Macroscopic : considerable, reddish brown. Microscopic : uric acid, oxalates, blood, and pus may be present.
<i>Remark.</i>	Intermittent hydronephrosis sometimes occurs with movable kidney (Osler).

II. SYSTEMIC DISEASES.

<i>Name.</i>	1. Diabetes mellitus (characteristic urinary changes).
<i>Synonyms.</i>	
<i>Color.</i>	Pale, clear, almost colorless. Often frothy on surface, due to fermentation.
<i>Odor.</i>	Sweetish.
<i>Quantity.</i>	Greatly increased, 2500 to 14,000 c.c. in twenty-four hours.
<i>Reaction.</i>	Acid.
<i>Spec. Grav.</i>	Usually high—1030 to 1048; may be low, however; very high specific gravity should arouse suspicion of fraud.
<i>Albumin.</i>	Either absent or a trace.
<i>Sugar.</i>	Present, from a fraction of 1 per cent. to 10 or 12 per cent. ; increases on standing.

Urea. Increased.
Sediment. Macroscopic : variable in quantity and appearance.
 Microscopic : oxalate of lime and uric acid crystals, yeast cells, and fragmented and whole epithelial cells.

Name. **2. Diabetes insipidus** (characteristic urinary changes).

Color. Colorless.

Odor. Odorless.

Quantity. Very greatly increased—5000 to 30,000 c.c. in twenty-four hours.

Reaction. Acid or alkaline.

Spec. Grav. Low—1001 to 1008.

Albumin. Absent ; rarely a trace.

Sugar. Absent.

Urea. Diminished.

Sediment. Macroscopic :
 Microscopic : few epithelial cells.

Remark. The total solids may not be reduced. The terms glycosuria and polyuria are sometimes used incorrectly as synonyms for the foregoing. Sugar from any cause in the urine is termed glycosuria, and is not necessarily due to diabetes. Any marked increase in the amount of urine above the normal is termed polyuria, and is therefore applicable to any increase due to other causes than diabetes. Anuria, the opposite of polyuria, occurs in a number of conditions, but is distinctive of none.

Name. **3. Rheumatism.** This is frequently associated with excessive discharge of crystals of uric acid and oxalate of lime and amorphous and crystalline urates, or the urine has the characteristics of chronic parenchymatous or interstitial nephritis.

Name. **4. Gout.** This is frequently associated with excessive discharge of crystals of uric acid and oxalate of lime and amorphous or crys-

Remark. talline urates and the urine has the characteristics of chronic interstitial nephritis. There occasionally occurs in rheumatism and gout, as well as independent of these diseases, casts of amorphous urates, which must not be confused with the coarse granular casts. *The urate cast, beyond indicating the presence of urates, has no pathologic significance.*

Name. **5. Acute Infectious Diseases,** scarlet fever, diphtheria, measles, etc.; these are commonly associated with active congestion of the kidneys or acute parenchymatous nephritis. Anuria not infrequently occurs in these conditions. In typhoid fever, measles, and miliary tuberculosis, the Ehrlich diazo reaction is frequently given. In the first of these the typhoid bacillus may be recovered from the urine many weeks after the disease has subsided. It should therefore be remembered that the urine is a lively source of infection. The kidneys should be freely flushed and the urine disinfected.

Name. **6. Heart and Lung Diseases.** These are commonly associated with passive congestion of the kidneys or chronic interstitial or parenchymatous nephritis.

III. SPECIAL CONDITIONS.

Name. **Urethral Fever.**
Synonym. Sound fever, stricture fever (Holt).
 Nothing characteristic except occasionally a trace of albumin and high colored urine.

Name. **Renal Colic.** Nothing characteristic in the urine except possibly greatly diminished quantity or suppression (anuria) followed by hematuria; occasionally a trace of albumin. See *Renal Calculus*.

Name.	Hematuria.
Synonymy.	<i>Blood-corpuscles</i> in the urine.
Color.	Same as hemoglobinuria. See <i>Sediment</i> , however.
Odor.	Nothing characteristic.
Quantity.	Normal.
Reaction.	Usually alkaline.
Spec. Grav.	High—1025 to 1035.
Albumin.	Present.
Sugar.	Absent.
Urea.	Normal or increased.
Sediment.	Macroscopic: upper portion of specimen usually less tinged than that seen in hemoglobinuria. Heavy, granular. Microscopic: numerous red blood-cells, leukocytes, granular, hyaline, and blood-casts. Whole and fragmented epithelium.
Name.	Hemoglobinuria.
Synonym.	<i>Blood coloring-matter</i> in solution in the urine, <i>i. e.</i> , dissolved out of the blood-corpuscles.
Color.	May be blood red, dark yellow, or dark red. This is spoken of as smoky. Always opaque.
Quantity.	Normal.
Reaction.	Acid or alkaline.
Spec. Grav.	High—1020 to 1030.
Albumin.	Present in abundance.
Sugar.	Absent.
Urea.	Increased.
Sediment.	Macroscopic: heavy, granular, varying degrees of pigmentation. Microscopic: few hyaline, granular casts, epithelial cells, and much detritus. A few red blood-cells. Blood pigment shown by proper tests.
Name.	Jaundice. The urine in this condition is very dark, almost black, opaque, and if made to froth, shows a bright yellowish tinge in froth. Bile may be demonstrated

by definite tests, otherwise nothing characteristic.

Name.

Pigmentations of Urine. These various colorings of the urine occur under the influence of certain drugs, and are mentioned here only to direct the reader to special investigation in reference thereto.

Retention. Urine held in the bladder.

Suppression. *Synonyms,* anuria, cessation of renal function. These terms will be found under the pathologic conditions producing them.

The more unusual and inconstant constituents, as indican, leucin, tyrosin, etc., and the conditions associated with them, are omitted from this list for the reason that it is thought better for the reader to consult the sections treating especially of these.

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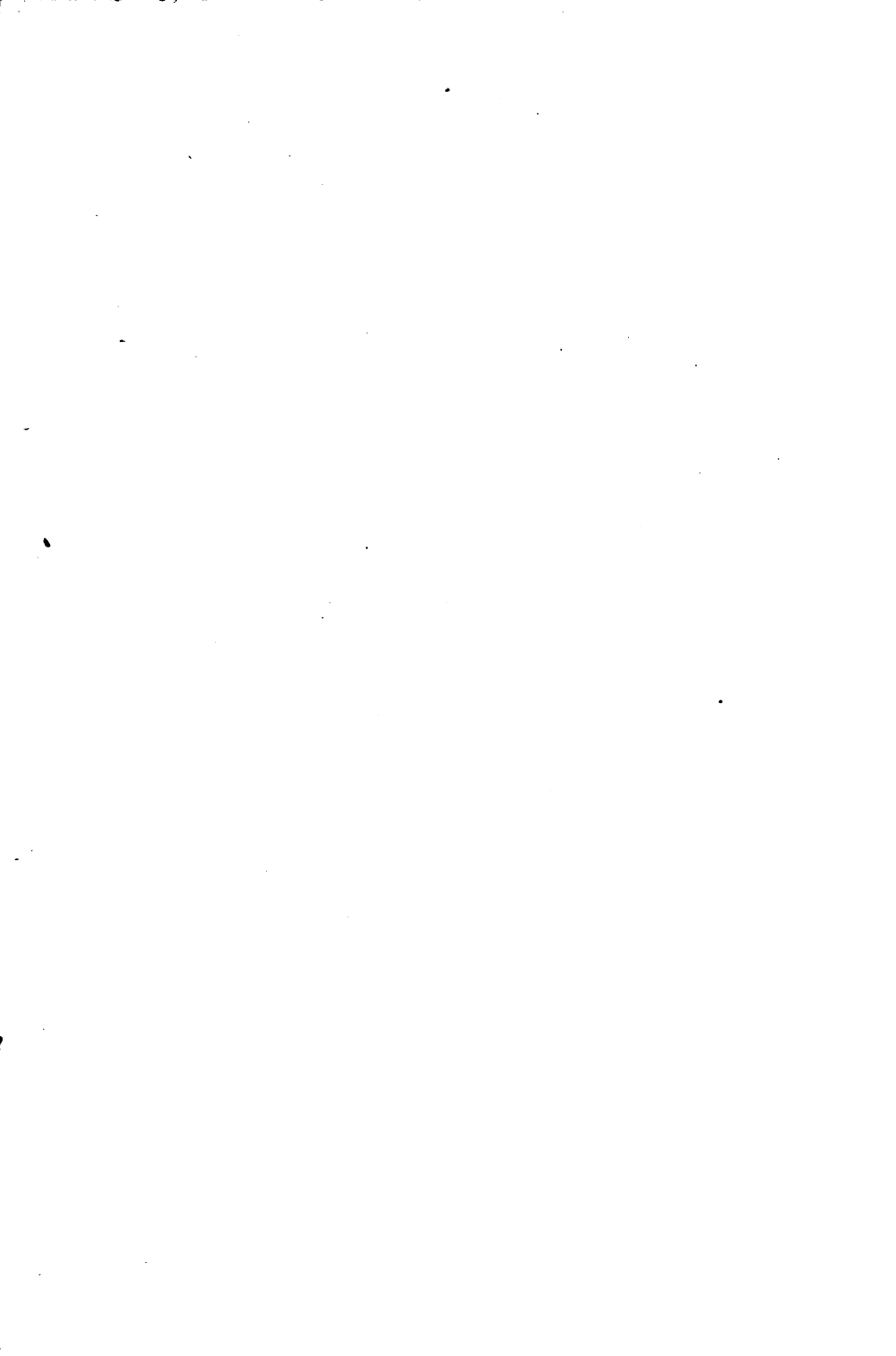
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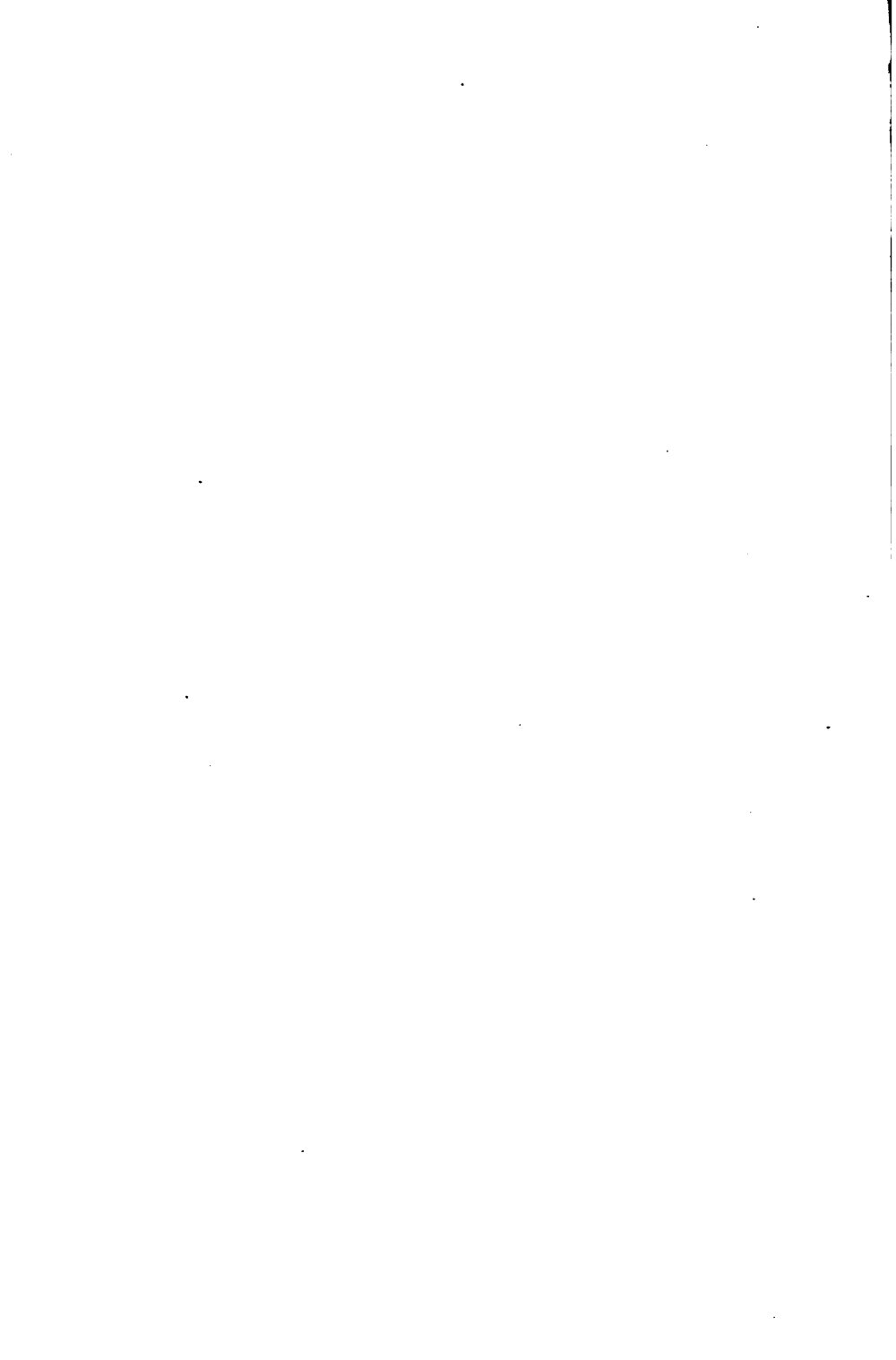
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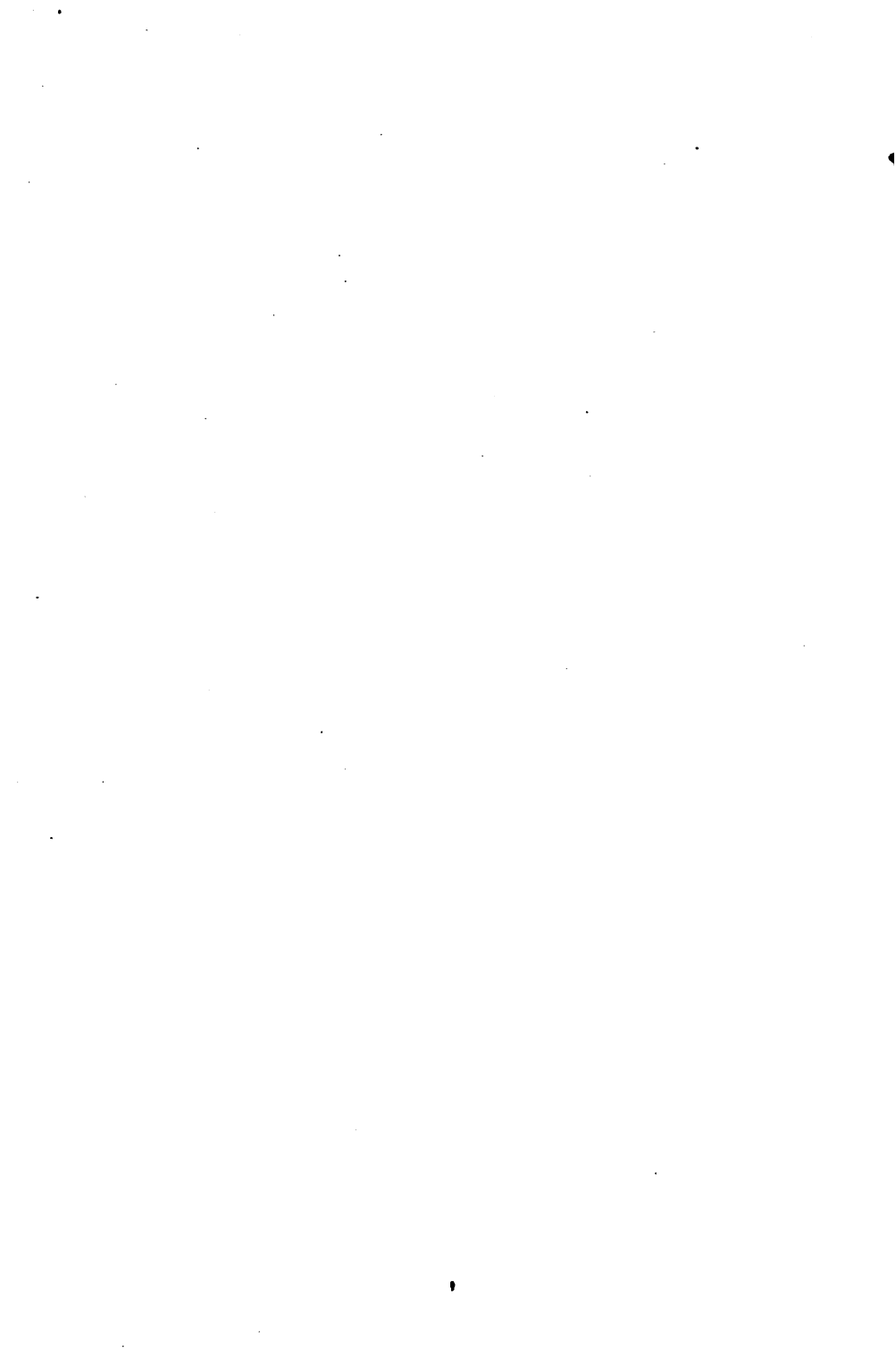
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